

Amber light for genetic manipulation

THE report of Lord Ashby's working party (Cmnd 5880, HMSO, 26p) makes it seem more likely than not that biological research involving the "manipulation of the genetic composition of micro-organisms" will continue in Britain, but in an atmosphere which takes account, chiefly through the laying down of proper laboratory procedures, of the possible attendant hazards. Stated like that, it all sounds eminently reasonable, but just what will happen next, and how hazard-proof can the eventual safety arrangements hope to be?

The working party finds that the possible benefits which may accrue from the continuation of such research are great—for example the 'mass production' of genes responsible for promoting the synthesis of insulin and the like. The report also describes the possible hazards (and says that there is always the possibility of these emerging in a completely unexpected way). Weighing the two sides up, the conclusion arrived at is that provided the precautions mentioned in the report are "taken by trained and disciplined workers, the potential hazards could be minimised, and the potential benefits would amply repay the trouble and expense which the precautions would involve". That is the report's thesis in a nutshell.

The real question is whether it is possible to impose from scratch, on scientists and technicians who have not been used to them, the disciplines of institutions that deal on a day-to-day basis with pathogenic organisms—those responsible for cholera and rabies, for example. It is certainly more difficult to persuade a whole laboratory that it must adopt new and time-consuming procedures than to convince an individual scientist, joining, say, a public health laboratory, of the necessity for all the precautions he finds there. The matter is made more difficult because the cholera vibrio is dangerous, period, whereas many of the genetic alterations brought about by cutting and joining pieces of bacterial DNA, or tampering with viruses, may or may not be harmful to humans. Alas, it would be all too human to pay only lip service to the best of precautionary arrangements, for that very reason. It is a salutary thought that even laboratories dealing with the smallpox virus are not immune from accidents of the type which caused the smallpox outbreak in London in 1973.

Containment procedures are, of course, important in the context of microbial genetics, but should they be the first line of defence as the working party suggests? The working party discusses several back-up procedures, including epidemiological studies on people carrying out work on genetic engineering of micro-organisms, and mentions ways in which, without prejudice to the science, at least some micro-organisms of interest could be made safe from the start. One possibility is to "dis-

arm" potentially dangerous plasmids or bacteria by altering their genetic composition a bit more so that, for example, useful but possibly unsafe strains of *Escherichia coli* could not survive in the human gut because the chemical environment or the temperature were wrong. (Those dealing with the most dangerous 'conventional' pathogens do not, of course, have that option open to them.) This is potentially a much more watertight approach than: a micro-organism may be able to survive in the human gut, it could be dangerous, so it should never get there.

Other ways of thinking have been canvassed—for example that the most dangerous experiments should be done in a few secure establishments—but where is the border line, what is a 'dangerous' experiment? In any case there would probably be scant support for the idea that a major growth area in the biological sciences should be confined to a few selected laboratories.

Much of the working party's report is, rightly, devoted to the risks to humans that might attend genetic manipulation of bacteria. But there is, as the report acknowledges, an "analogous risk" from similar work on animal and plant diseases. Here containment is very much the order of the day, is accepted as such by those involved, and in the case of some dangerous organisms is already backed up by legislation.

Assuming that the main thrust of Lord Ashby's report is accepted, what will happen next? The document is for discussion, and discussed it will be, especially at the conference at the end of this month arranged by Professor Paul Berg, one of the instigators of the National Academy of Sciences moratorium. The institution of a code of practice was recommended by the Ashby working party, but it was not within its brief to come up with chapter and verse. In Britain, there is already a body that might fit the bill—the Godber Committee, which is examining safety procedures in the laboratory use of dangerous pathogens following the 1973 smallpox outbreak. This working party is expected to report soon. (One of the suggestions made in the Ashby report is that a biological safety officer should be appointed wherever the experiments in question are to be carried out. That would not be sufficient, and in practice a system of inspection would be the only real way of keeping things up to scratch.)

Whatever happens, the Medical Research Council has stated that it will allow the research to go on, but accompanied by containment precautions of its own if nothing is imposed from above. The universities (through the University Grants Committee) have also taken the points made in the report and are examining in detail the situation as it affects them with a view to setting up their own rules. □



Immigrants at Lod airport. Photo Enkka.

high energy particle physics which can only be tackled on a multinational or superpower budget, are clearly ruled out); they have to cope with a whole new system of funding projects and buying materials. Coming from a system where new graduates are directed into industry or research, they have no experience of such basic procedures as interviews and applications. Some are distressed by lack of 'status'—the new immigrant working in an Israeli academic institution has his salary paid for the first two years by the government, a situation which bypasses the current cut-backs and redundancies necessitated by Israel's economic situation, and also somewhat relieves the budget of the department concerned, releasing funds for equipping the immigrant scientist for his research. During these two years, however, he is essentially on probation and his appointment still awaits confirmation. Coming from the Soviet system, where the status of a scientist is extremely high, some scientists seem to find difficulty in accepting, even temporarily, a position in which they have a salary but no status.

It is easy, of course, to exaggerate the importance of such difficulties—and the comments of the scientists themselves must be treated with a certain discretion. Many have, after all, chosen to come to Israel since they feel that the Soviet system is not conducive to academic freedom. One physicist in particular, who, when interviewed about his reactions to working in Israel, took advantage of the opportunity to offer extensive criticism of the absorption process, and, indeed, of the Israeli economy and political system, concluded by saying that he loved living in Israel "for here I can criticise as much as I like." Many criticisms seem to arise from a sense of enthusiasm. Scientists settled in a job have anecdotes of friends awaiting absorption, who want "to use their resources and experience" for their new country. More organisation is needed, they urge. "Here, in Israel, all things go slowly."

To hasten the process, the Israeli government set up, at the end of 1973, a Centre for Absorption in Science, under the auspices of the Ministries of Labour, Absorption, and Finance, and of the *Sochnut* (Jewish Agency). It is staffed by seven 'professionals'—themselves scientists who felt that their real métier lay in administration rather than research—assisted by a small secretarial staff. Doctor Uri Horowitz, Director of the Centre, explains their task of that of "intervention to get the scientist into the centre of his own communications network", in other words, to familiarise him with the whole job-finding routine and to introduce him to the various contacts he will need in his professional

Coping with an exodus

The Soviet government's refusal to accept the conditions of the trade agreement with the United States has left in an equivocal position the Russian Jews waiting to leave their country. Meanwhile, Israel carries on with a reception programme which has handled 100,000 immigrants since 1970. Vera Rich reports from Tel Aviv.

THIS month, the 100,000th Jewish emigrant to leave the Soviet Union since 1970 arrived in Israel. In spite of Soviet claims that 98.5% of all Soviet Jews wishing to emigrate are allowed to do so, there is known to be a backlog of some 140,000 applicants still awaiting permission to leave. Although the effect on Jewish emigration of the recent Soviet rejection of the US-Soviet trade agreement cannot yet be gauged, there is no indication that it will lead to any official change of policy—indeed, a statement made by Colonel Alpachnikov of OVIR (the visa department of the Ministry of the Interior) on January 17, the day after the rejection of the agreement was announced, suggests that the policy will remain as before, one of frustrating delays and harassment, but with no absolute bar to emigration. The flow of Soviet Jews arriving in Israel may well slacken off for at least a time, but so far there are no signs of a total halt.

The problems of absorbing these immigrants into the Israeli economy are well known, and have become, indeed, the subject of a number of wry Israeli jokes ("What does one do with 50 professors of Russian philology?"). The

situation is aggravated by the fact that, on the one hand, some of the newcomers have a higher education and wish to continue to work in a learned profession, and, on the other hand, this higher education is sometimes so extremely specialised that even the smallest change of employment amounts virtually to a change of métier (one young engineer, at present going through the absorption process, quoted his first degree as having been in "Strength of Materials of the Aircraft Construction Industry"). And even those scientists who are able to be absorbed directly into Israel's higher education and research system may well find their practical problems only just beginning.

The problem, for a recently arrived scientist from the Soviet Union is largely one of adjusting his or her mental attitude from that of state-run, state-controlled research, to what has been described as the "anomic situation" of Israeli academic life. For the first time, they are faced with the problem of justifying their proposed research on an economic basis. It is not only the problem of what Israel can afford to pay for (some subjects such as

life. Although the centre does not deal exclusively with immigrants from the Soviet Union, they do, in the present circumstances, form the majority of its clients. The centre aims to promote the good of the scientist rather than the product, and, accordingly, the new immigrant is presented with a set of forms to fill in, in his own language, with questions structured according to what he expects in an enquiry of this kind—even if, in certain cases, the information is irrelevant to the needs of the centre. Having completed his forms, the immigrant is interviewed and questioned about the work he would like to do. He is then asked to write a feasibility study of his proposed project, with the emphasis on the part he himself would play in it. (It seems that some idealists have submitted projects which they feel would benefit Israeli science, but have forgotten to write in a job for themselves.) After completion of this study, the centre then discusses ways and means of fitting the scientist and his proposed project into the existing structure of Israeli science.

In general, the impression gained from interviews with scientists who have been absorbed is that remarkable efforts have been made to keep them within their same general discipline. Some of the cases of "change direction" quoted prove on investigation to be nothing more than change of employment. Thus the Kinneret research station (attached to the Technion) has absorbed five immigrants from the Soviet Union, only one of whom is a limnologist, the other four being physicists and chemists, who are, however, still working in their original fields, although now on the physics and chemistry of lake water. The need for 'retraining' often referred to may cover nothing more than the need of a computer expert to learn a new programming language. Some scientists arrived with a choice of interests, and have found their subsidiary interests in Russia can be their main interest in Israel. The main problems seem to arise with those who qualified not in one of the big state universities, but in a small Institute of Higher Education, where the subjects taught are extremely narrow and the graduate is, by western standards, more an applied technologist than a scientist. For some of these, retraining becomes, in effect, a chance to bring one's education up to full academic standard, and to advance from being, say, a computer technician, to a systems programmer.

Even for the best qualified scientists, there is, on arrival in Israel, generally a backlog of study to be made up. Under the current Soviet practice towards academic would-be emigrants, application for a visa is followed by a number of forms of harassment, including

dismissal from professional employment and the cutting off of one's telephone, which entails long and tedious journeys to arrange even the simplest details connected with one's hoped-for departure. If, as is often the case with scientists, the initial application is rejected, one may spend months or years as a "refusnik", with neither the opportunity nor the necessary time to keep up one's reading or think about one's own research. (The famous 'Sunday seminars' were started as an incentive to scientists in this position to attempt to keep up their work. On arrival in Israel, therefore, the scientist is not only faced with learning a new language, but with making up vast arrears of essential reading.

As far as possible, the universities do assist in the problem of learning Hebrew. The Ben-Gurion University (Beer Sheva) employs its immigrants on a kind of sandwich-course basis, half their time being devoted to studying Hebrew. Tel Aviv University, which has absorbed some 280 immigrants (including postgraduate students) has its own *ulpan* (Hebrew school for immigrants). The backlog of reading presents a greater problem—and is one cause, in the case of long term "refusniks", of scientists deciding not to continue in their chosen profession but to turn to some other field of activity like administration. Once an effort is made, however, and the reading started, the greater and more rapid availability of journals, and the chance to correspond freely with colleagues throughout the world, begin to show results and the information gap is rapidly reduced.

This freedom of correspondence, and the chance to work on one's own initiative, instead of as part of a closely controlled team subject to state direction, are seen by the scientists as the greatest benefits of their new academic life. The universities in their

turn find the immigrants from the Soviet Union a source of new vitality and expansion. The Pure Mathematics Department at Tel Aviv University was "virtually non-existent" until the wave of Soviet immigrants arrived; now it is acquiring a considerable reputation and the university is hoping to expand its Astronomy Department in a similar manner. The Ben-Gurion University, having absorbed seven Soviet pure mathematicians into senior teaching posts, is making tentative plans (subject to funding) to develop an Institute of Applied Mathematics that could take, initially, about 20 or 30 applied mathematicians. This university also offers considerable scope to Soviet scientists, especially those dealing with the more applied branches of physics and chemistry, in its raw materials programmes, and also to agronomists in its desert research programme. The vast scientific complexes of the Technion and the Weizmann Institute likewise still seem to offer considerable possibilities for absorbing further immigrants. The main problem remains that of funding—but Israel has a long history of foreign endowments for academic institutions and, in spite of economic difficulties, one feels that in the long run the money will be found.

Although an upper limit to absorption must at some time be reached—if only when the whole state of Israel is transformed into one vast academic campus, coterminous with the state frontiers, with the entire population either engaged in academic research or providing the ancillary services to keep the academics alive (the "think-tank of the western world", as one scientist described it)—that time has not yet come. And in the meantime, Israel provides a fascinating case study of the problems of transition from state-controlled research to one based essentially on personal initiative. □

Immigration officials check the papers of Viktor Polski, on arrival in Israel.



international news

SSTs: it's an ill wind . . .

by Colin Norman, Washington

THE United States Department of Transportation last week made public the results of a three-year investigation (costing \$20 million) of the likely environmental effects of supersonic aircraft flying in the stratosphere. The event was a mixed blessing for the financially beleaguered Anglo-French Concorde project.

First, the study reached the conclusion that the present number of supersonic passenger aircraft (SSTs), together with those now scheduled to enter service, would cause climatic changes which will be too small even to measure. But that conclusion comes as no surprise since sales of Concorde have been so dismal that a maximum of only 30 SSTs are expected to be flying by the late 1970s (about 16 Concorde and 14 Soviet TU-144s)—a number which is unlikely to represent a threat to anything except Britain's balance of payments.

More ominous news from Concorde's point of view is that the study has essentially confirmed the validity of the much publicised theory that a large fleet of SSTs, equipped with present-day engines, could damage a layer of ozone in the upper atmosphere which shields the Earth from ultraviolet radiation. Consequently, if the size of the SST fleet increases significantly, serious environmental damage could result unless the aircraft are fitted with cleaner engines, the study indicates. The argument about the effect of SSTs on the environment is therefore unlikely to be stilled by this report.

Called the Climatic Impact Assessment Project (CIAP), the study was ordered by Congress after fears about the effect of SSTs on the ozone layer had played a key role in the Senate's momentous decision in 1971 to terminate development of an American SST. The basis for those fears has been hotly debated ever since.

The theory, put forward in 1970 chiefly by Dr Harold Johnston of the University of California, was that oxides of nitrogen, spewed into the stratosphere by jet engine exhausts, would upset the delicate chemical equilibrium of the ozone layer, destroy a part of it, and cause an increase in the amount



Concorde, accelerating at 35,000 feet

of short-wavelength ultraviolet radiation reaching the Earth's surface. One consequence would be to increase the incidence of skin cancer—an emotive topic if ever there was one.

Although it has turned out that Johnston's calculations (*Science*, **173**, 517; 1971) overestimated the effect of SSTs on the ozone layer, chiefly because they were based on erroneous government projections of the size and character of the SST fleet in the mid-1980s, the CIAP study has not refuted Johnston's theory.

The CIAP's report states that although there is still some uncertainty in parts of the calculations, a cause-effect relationship between operation of SSTs in the stratosphere and increased ultraviolet radiation reaching the Earth's surface has been firmly established. The extent to which a given increase in ultraviolet radiation will increase the incidence of skin cancer is more uncertain, the study suggests, but again, a link has been established.

Proceeding from the undisputed assumption that pollutants injected into the stratosphere can stay there for periods of three years or more, the CIAP report argues that the problem is not confined solely to supersonic aircraft since some wide-bodied subsonic jet airliners now fly in the lower stratosphere.

But how great is the effect? According to calculations presented in the report, the fleet of subsonic jets now in operation, which includes about 7,000 aircraft operating in the lower stratosphere, is probably depleting the ozone layer by about 0.1% over the next ten years, and the 30 or so SSTs which will be flying in the next few years can be expected to add a further 0.1% depletion. Those effects are, however, much too small to be detected since the minimum change in ozone level that could be picked up by a global monitoring

system, operating daily for 10 years, is about 0.5%, the report notes.

According to Dr Alan J. Grobecker, Director of the CIAP, it would take about 125 Concorde-class SSTs flying in the stratosphere for between four and five hours a day to reduce the ozone layer at the minimum detectable rate. Grobecker pointed out in an interview last week, however, that if demand for SSTs picks up in the early 1980s, it is conceivable that 70 Concorde and a similar number of TU-144s could be in service by 1990, which would cause at least a measurable change in the ozone layer.

To put those figures in perspective, however, the study points out that the ozone layer is about three times thicker at the poles than it is at the equator, so that there is considerable natural geographic variation in ultraviolet flux reaching the Earth's surface. Moreover, the distribution of ozone at a given locality can fluctuate by up to 25% from day to day. Set against those natural variations, the change likely to be caused by the SST is relatively minor. But the study notes that a small change in the average amount of ultraviolet radiation around the world may result in a relatively large absolute number of skin cancers. And that is not a prospect which government regulators can take lightly.

Thus, if the size of the SST fleet increases, the report recommends that SST engines should be redesigned to reduce the emission of oxides of nitrogen. Studies conducted by NASA have indicated that emissions of oxides of nitrogen from Concorde-type engines could be reduced by a factor of six without sacrificing performance, but it would cost about \$50 million and take up to 15 years. A more radical engine redesign, to reduce emissions by a

factor of 10 or more, would take up to 25 years, the report notes.

"If it can be made clear, very soon, that such reduced emissions will be required of aircraft of all nations, and if design to reduce those emissions is incorporated promptly into ongoing engine development, ozone reduction could then be kept near the current reduction due to aircraft alone (less than 0.1%) . . . for fleets up to 4,000 747-class subsonics at 11 km (38,000 feet) or 1,000 at 13 km (43,000 feet), and for 150 Concorde/TU-144-class SSTs", the report calculates. Over the longer term and with the more radical engine redesign, "stratospheric air travel could expand as a function of demand without affecting the environment adversely, in keeping with deliberate calculable standards enforcement".

The key to those sanguine predictions is early development of international standards to force SST emissions to be lowered. But, given the tortuous history of attempts to regulate emissions from automobiles in the United States, that will be no easy task. Nevertheless, the report argues that "the process of establishing and meeting standards should start now because of the long lead times involved".

As for the biological effects of increased ultraviolet radiation resulting from the operation of SSTs in the stratosphere, the CIAP report states that the percentage increase in ultraviolet flux is about twice the percentage decrease in the lower ozone layer. Thus, the smallest detectable depletion of the ozone layer—0.5%—would increase ultraviolet flux by about 1%. Though the effect of such an increase on the incidence of skin cancer is not readily calculated, the report notes that an upper level effect can be calculated by postulating that ultraviolet radiation plays the only role in development of non-melanomic skin cancer. An increase of 1% in ultraviolet flux would therefore cause an increase of 1% in the number of skin cancers.

In addition to those suggestions, the CIAP report also notes that another possibly serious problem with a large number of SSTs is that sulphur compounds in jet fuel could end up as solid particles of sulphuric acid floating in the stratosphere. The effect of that would be to filter out some of the solar energy reaching the Earth, which in turn could reduce the mean global temperature. Although it would take a very large fleet of SSTs to produce a detectable effect, the report notes that a difference of only a fraction of a degree would cost hundreds of millions of dollars in crop losses. The report therefore suggests that consideration be given to regulations to force high flying aircraft to use low-sulphur fuels. □

India's space programme

from Narender K. Sehgal, Jullundur

INDIA's modest space programme is facing rough weather. Until about two to three years ago, things were going fairly well and were not too far behind schedule. Then generally difficult economic conditions, coupled with inflation and rising costs, began to take their toll. Funds have since been scarce and slow in coming, so important projects are running late, and some have had to be curtailed or postponed. Others face an uncertain future as decisions on them are kept pending.

Space research formally began in India with the creation of INCOSPAR (the Indian National Committee for Space Research) in the Department of Atomic Energy (DAE) in 1962. The programme started off with the launchings of sounding rockets from the Thumba Equatorial Rocket Launching Station near Trivandrum, mainly for meteorological and ionospheric investigations in the upper atmosphere. Following administrative and organisational changes over the years, ISRO (the Indian Space Research Organisation), a body under the Department of Space, took over responsibility for all matters relating to the country's space programme in 1969.

From small beginnings in 1963, when the Thumba station became operational, the programme has grown steadily and now covers a whole range of activities relating to rockets and satellites. The Space Science and Technology Centre (SSTC) near Thumba is the research and development unit of ISRO and is responsible for (1) work on systems and their components required in space research; (2) carrying out pilot production of equipment resulting from such work; and (3) developing an indigenous satellite launch capability not only linked to scientific exploration but also to communications, meteorology and remote sensing.

Besides the Thumba station and the SSTC, several other establishments are also engaged in work connected with different aspects of the research programme.

According to original plans, the high spot of India's space programme—the orbiting of an Indian satellite taken up and Indian rocket launched from Indian soil—should have been attained during 1974. By the end of 1972, however it had become quite clear that work on the project was running behind schedule and that the launch would not be possible before 1977. Apparently, the delay was mainly because of difficulties with the satellite

launch vehicle project, since in early 1973 it was announced that an Indian satellite would go into orbit in the middle of 1974 atop a Soviet rocket launched from within the Soviet Union. This was to be a much heavier payload than the one planned for an Indian launch. Though good progress has been reported from time to time on the satellite part of this latter project, the launch, first postponed to the end of 1974, has now been rescheduled for sometime during 1975. A satellite prototype was reported sometime ago to have undergone successful tests both at Bangalore and in the Soviet Union. The small delay in launching could be because of either or both of budgetary difficulties and technical reasons, though there are certainly some grounds for thinking that the reasons lie elsewhere.

Sources at the Department of Space and ISRO have flatly denied a report in a British Interplanetary Society journal that India had agreed to make port facilities available to Soviet recovery ships in the Indian Ocean in return for Soviet launching of India's first satellite (weighing about 300 kilograms).

It is acknowledged a request for port facilities, for space tracking and recovery ships, was in fact received from the Soviet Union sometime in 1973. But the sources maintain that no decision has yet been taken in this regard and that this has nothing to do with the Soviet Union's agreement to launch the satellite, the two being entirely separate matters. It is believed that Indian scientists have been invited to instal research instruments in future Soviet space vehicles to the Moon and other planets, but the matter has yet to be discussed and finalised. A joint meeting of the space officials of the two countries is to take place in February this year for discussions on collaboration in space research.

Meanwhile, the Prime Minister recently told a consultative committee meeting that the original cost estimate of Rs15.6 crores sanctioned in 1973 for the satellite launch vehicle project would probably need to be escalated. "Apart from the increased cost, the special circumstances prevailing during the past two to three years have created difficulties in the way of speeding up the progress of the project", it was said. "Barring unforeseen problems, it is possible to achieve a satellite launch in 1978", the committee was informed.

The launch vehicle project is aimed at developing a four-stage rocket to fire a 40-kilogram satellite into orbit 400 kilometres up.

Full allocations of funds for the projects of the Department of Space have not been forthcoming, and this has resulted in an adjustment of priorities. □

Nuclear fuel plant mystery

by Colin Norman, Washington

A TECHNICIAN employed at a nuclear fuel fabrication plant in Oklahoma died in an automobile accident on November 13 last year when her car careered off a highway and struck a concrete culvert. The crash has brought national publicity to a bitter dispute over safety standards at the plant and sparked off a string of bizarre allegations which could reverberate for years.

When the accident occurred, the technician, Karen Silkwood, was on her way to meet an official of the Oil, Chemical and Atomic Workers' Union (OCAW) and a reporter from the *New York Times*, to discuss charges of lax safety precautions at the plant and allegations of faulty manufacture of plutonium fuel rods. Police investigators ruled out foul play and said she probably fell asleep at the wheel, but the union hired an automobile crash expert who concluded that she may have been forced off the road by another car. The matter is now under investigation by the FBI.

Although that event was mysterious enough, a series of reports by the Atomic Energy Commission (AEC) which investigated Miss Silkwood's charges have added yet another element of mystery to the affair.

Miss Silkwood and officials of OCAW launched the AEC investigations when they met AEC officials on September 27 last year to give details of 39 separate violations of safety regulations

which they alleged had taken place at the fuel fabrication plant. Operated by the Kerr-McGee Nuclear Corporation at Crescent, Oklahoma, the plant manufactures plutonium fuel rods for the Fast Flux Test Facility, an experimental breeder reactor installation being constructed in Washington State.

In the meantime, Miss Silkwood discovered on November 5 that her hands were contaminated with plutonium after she had been working with a glove box handling plutonium samples. She was decontaminated and found to be free from plutonium when she left the plant later that night. But when she reported for work the next morning, her hands, forearm, neck and face were again found to be contaminated with plutonium.

Then, on the morning of November 7, a nasal smear taken when she reported for work indicated a high level of plutonium contamination yet again, and urine samples she had collected over the previous two days were also found to contain significant levels of the substance. A subsequent check of her apartment led to the discovery of microscopic amounts of plutonium in the bathroom, kitchen and refrigerator, and her flatmate was also found to have two spots of low level contamination on her skin.

An investigation of those incidents was launched by the AEC immediately, but a report released by the commission last week simply adds to the confusion. The AEC investigators found, in short, that Miss Silkwood's contamination "probably did not result from an accident or incident within the plant",

and that the plutonium discovered in the urine samples "was not present in the urine when it was excreted". Finally, they suggested that Miss Silkwood "did ingest some plutonium on or about November 7, 1974".

The essence of those findings is that the AEC believes that Miss Silkwood may have contaminated her own skin, swallowed a tiny amount of plutonium and added plutonium to her urine samples. No explanation is offered of the reasons for taking such action, although others have speculated that she may simply have wanted to draw attention to the allegations of lax safety precautions at the plant. If so, self-contamination with plutonium seems an incredibly drastic step to take.

The Oil, Chemical and Atomic Workers' Union has sent the AEC's report to some scientists for review, on the basis of which Mr Anthony Mazzocchi, the union's Washington representative, said in a report to Kerr-McGee's union members last week that because Silkwood had a high respect for plutonium, "it is inconceivable to us that she would contaminate herself or her apartment deliberately". He suggested that "there is enough evidence to cause an investigation to be commenced into the possibility that Silkwood was intentionally contaminated by sources unknown to her", and called for the AEC to reopen its investigation.

Meanwhile, the AEC investigated the other 39 allegations of safety violations raised by Miss Silkwood at the September meeting, and came up with some evidence of inadequate precautions. The report, however, largely exonerated the plant management and the AEC termed most of the incidents minor.

A close reading of the report, however, indicates that even though some of the allegations may have been exaggerated, the AEC's findings still paint a fairly dismal picture. Take, for example, the allegation that there is a turnover of 60% of the work force at the plant each year. The claim, says the AEC is not substantiated because the actual turnover is 35%, which is still an amazingly high figure. Similarly, the AEC says that the allegation that 80% of the work force has less than two years of experience is incorrect. The actual figure is 62%.

● In Britain, the possible dangers of working with plutonium have been highlighted in the past few weeks by reports that the health records of workers at the British Nuclear Fuels Windscale works showed a small but significantly higher incidence of leukaemia than might be expected. Although the statistical validity of these results is still being debated, at least one union is undertaking an investigation into the situation. □

How do you get over the problem of calcium loss, particularly from the knee joints, during long space voyages? Recruit double amputees for astronauts, says Dr John M. Vogel, a specialist in nuclear medicine, working at the University of California at Davis.

As nobody has yet found a successful means of preventing calcium loss at the knee joints during periods of prolonged weightlessness, the Californian team is seriously investigating the next best thing: dispensing with knees altogether. As there are people around who have already lost their legs, why not make use of them as space pilots?

There could be many benefits from having legless astronauts. It might be possible, for example, to carry more of them in a given size of spacecraft than of the conventional biped astronauts employed so far. Double amputees would also presumably eat less food, and breath less air; and, being more compact, would probably find the cramped living conditions of

a spacecraft more comfortable than would two-legged astronauts. And the use of artificial legs instead of real ones during space walks or sorties on to planetary surfaces may prove a positive advantage. Not requiring the protection of a space suit, such legs would be in their natural environment in space rather than being less useful than real legs, as they seem on Earth. And as extravehicular activity would be carried out largely with the help of machinery anyway, with planetary 'buggies' and the like, the fewer human limbs to cater for, the better.

Of course, this whole philosophy could get out of hand. One could imagine armless astronauts ultimately, or perhaps, as medical science advances, even more bizarre developments. The Californian team is making its study in preparation for a possible voyage to Mars, which would take about six months with present technology. So, double amputees watch out for the advertisements.

from Peter Goodwin

Two Israeli university presidents, Professor Yuval Ne'eman of Tel Aviv University and Professor Moshe Prywes of Ben-Gurion University of the Negev, have submitted their resignations in recent weeks. Each has his own reason: Ne'eman has accepted a top post in the Defence Ministry and Prywes has been put in charge of all Negev medical services (in addition to directing the operations of Ben-Gurion University's newly established medical school). But their decisions may also have been influenced by the increasing difficulties faced by all local university presidents, who are striving to meet costs that have risen by 30% to 40% in one year while income—from Israel's University Grants Committee and other sources—has remained virtually static or has even declined.

Their problems are further complicated by charges in the press that too much money is being devoted to higher education at a time when insufficient funds are available for primary and secondary schools. Much of the criticism is extraordinarily simplistic: comparisons are made, for example, between the sum spent on the education of a primary school child and the money spent on the training of a university student, without taking into account, among other things, the university's role as a research centre.

Also stemming from the current economic situation is the government request that Israeli professors either give up their sabbatical leave this year, or, alternatively, spend it at another local institution. Professor Amnon Rubinstein, of Tel Aviv University, believes the request is justified "in a year of military tension and economic crisis." His colleague at Tel Aviv University, Dr Benjamin Rulf, has, in contrast, received the idea very coolly. Rulf warns that overseas sabbaticals are an essential safeguard against "scientific degeneration."

It now seems that the professors who are supposed to go on sabbatical leave this year will be asked to remain in Israel, but that no binding regulations will be brought in.

Economies, meanwhile, are proceeding in other spheres. Classes at the Hebrew University, to give just one example, are ending this winter at 20.00 instead of 21.00 in order to cut down on lighting and heating costs. The university has also announced a freeze on the hiring of new personnel to fill vacant posts.

But such paring down around the edges will not be sufficient. Barring some drastic and unlikely increase in funds from either Israeli or overseas sources, large scale dismissals are inevitable. In fact, Tel Aviv University

has already stated that 400 posts will have to go—some through natural wastage, others through dismissals. Other universities are likely to follow suit.

● Research grants from overseas bodies continue to play an important role in keeping afloat Israeli institutions of higher learning. This is particularly true at the Weizmann Institute, whose scientists manage to obtain a good many grants in the face of fierce competition.

Among the recent grants to be announced at the Weizmann is one of \$146,000 from the National Cancer Institute of the US National Institutes of Health to Professor Leo Sachs for research into the carcinogenic properties of chemical compounds. It will help

Letter from Israel

from Nechemia Meyers

Sachs further develop his method, already adopted overseas, for testing suspect chemicals in tissue culture rather than in experimental animals.

German funds are as important a source of support of Weizmann Institute research as money from the USA, the traditional sugar-daddy of international science. The Minerva Fund, a subsidiary of the Max Planck Society, is alone providing DM5 million to finance 64 projects at the Weizmann Institute during 1975.

Industrially sponsored research at the Weizmann (and elsewhere) is growing, but still leaves much to be desired. Rehovot scientists, for example, have \$1.25 million in industrial research contracts, 70% from overseas companies and 30% from Israeli ones. Most agreements, negotiated by the Yeda Research and Development Company, involve plastics, chemicals or pharmaceuticals.

● Mr Issac Fleischmann, Director of the US Patent Office's Information Service, suggested during his recent visit that Israelis, in addition to developing their own processes and devices, should make a more vigorous effort to exploit those developed in other countries.

Fleischmann pointed out that relatively few patents have been issued in Israel, which means that hundreds of thousands of ideas protected elsewhere may be borrowed freely for use here. He found it strange that there are so few Israeli subscribers to the US Patent Office's *Official Gazette of Patents*, which each week carries a summary of

some 1,500 or 1,600 newly granted patents (about the number issued in Israel, to both local and overseas applicants, in the course of a full year).

● An (unpatented) Israeli development which should be of interest overseas is "Help Pollution", a game of the Monopoly ilk now being used as a teaching aid in high school biology classes. Created at Bar-Ilan University by Dr Uri Yoel, it is won by the player who is most successful in his attempt to prevent pollution of the Sea of Galilee, the country's main fresh water reservoir, which is facing a very real pollution threat from the inflow of municipal sewage and agricultural fertilisers, as well as from the rubbish left around its shores.

As in Monopoly, players acquire property around the lake and then try to increase its value. To do so they must prevent pollution of their land and of the lake by, among other things, investing money in anti-pollution measures. Uncertainty is added by accidents, such as DDT seeping into the water.

● While Israeli schools teach youngsters to protect their country's environment, Israel's national radio and television stations are playing a song which glorifies a man for inscribing his name on a public building. He is Baruch Jamili, a soldier in the 1948 War of Independence, who chose to write his name in tar on a water pumping station near Jerusalem. Seeing the name 25 years later, the song writer and singer Shlomo Artzi wrote "The Ballad of Baruch Jamili", which presented Jamili as a war hero and won the last Israel Song Festival.

The outraged Nature Reserves Association promptly issued a statement strongly attacking the song, which, it fears, will encourage non-heroes to inscribe their own names on public buildings.

Graffiti are not a new problem here, as witnessed by the inscriptions that have been left all over the area for untold centuries. But the signature of a Crusader knight on the wall of a dining hall in Sinai's Santa Katerina Monastery is history whereas a signature added by a contemporary visitor is regarded as vandalism.

A unique attempt to let the graffiti artists express themselves, and yet safeguard the environment, is being made at a scenic canyon in the Negev desert, near the Red Sea port of Eilat. Taking a positive approach, the local authorities have set aside a special slab of rock where visitors are invited to carve or write their names.

They are politely requested to leave the rest of the canyon untouched.

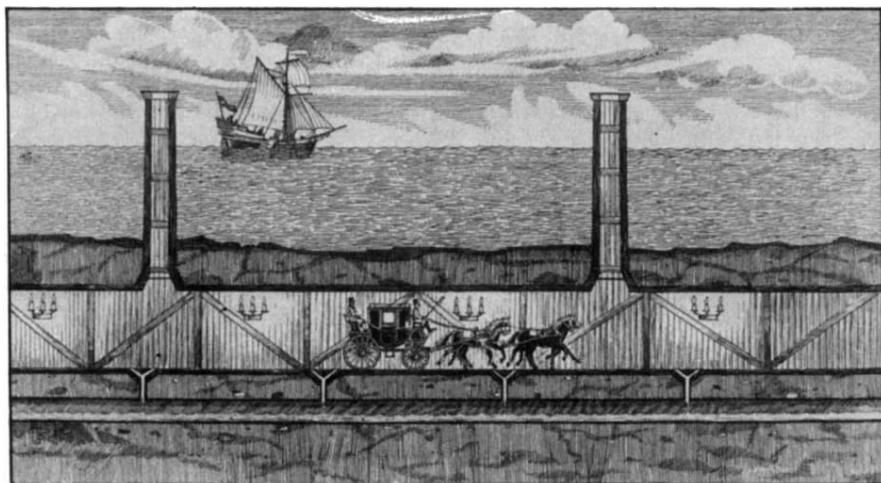
Corking the Chunnel

from Angela Croome

THERE have been complaints that the cancellation of the British-French Channel Tunnel Project last week by Anthony Crosland, Secretary of State for the Environment, was both hasty and premature. But this seems a point of view that is difficult to justify for a scheme that has been in the pipeline (as it were) for well over a hundred years. Indeed, a look at the 'on/off' history of this latest Chunnel plan (going back to the 1962 White Paper *Proposals for a Fixed Channel Link*) it is remarkable that the scheme survived the rejection in 1968 by both the British and French governments of the industrial proposals they had called for the year before. Rather than projecting an image of the 'white heat of technology', which was the watchword of the then Wilson government, the plan always had a gaslit and fustian air about it.

Tunnelling was above all a nineteenth century enthusiasm. It was indeed a technological innovation then—an extension of the techniques used in mining deeper and in some cases (as in Cornwall) under the sea. The Cornish engineer, Richard Trevithick, was called in to complete the first Thames tunnel (between Rotherhithe and Limehouse) in the early 1880s. With the aid of picked gangs of stout Cornish miners accustomed to working in a crouched position and in unbelievably horrible conditions he would certainly have succeeded had the money not finally run out only 200 feet from the far end. There is no trace of Trevithick's tunnel today. It took the Brunels, father and son, another 40 years to bring off the first Rotherhithe tunnel for wheeled traffic (now used by the London Underground). There were several cave-ins and a number of miners also died from the "tunnel disease"—a lung complaint attributed to the poisonous air in the workings. Many technological advances were made in completing "The Thames Tunnel" as the Brunel venture came to be called, including the special shield designed by Isambard Kingdom Brunel on the system of the burrowing shipworm *Teredo navalis* to hold the newly bored walls in place until lining was completed. But bridges increasingly superseded tunnels as a traffic link as the century progressed. And it is hard to see that there is any comparison at all in convenience, flexibility and 'passenger satisfaction'. The prospect of travelling not merely underground but underwater for the best part of an hour is a nightmarish prospect for many people.

Despite the long gestation period of



To Paris by carriage (with a gaslit and fustian air).

the Chunnel scheme, and the various assessments and reappraisals, the Cairncross review, the revised rail-link proposals and so forth, there has never been the sense of a real solution to the cross-channel traffic problem but rather a look of amateurish gimmickry. Has a serious study ever been made of a cross-channel bridge? Would it really be more vulnerable than a tunnel? Some years ago Desmond King-Hele put forward the virtues of a channel dam—and not entirely as a *jeu d'esprit*.

France is better linked to Britain than is any other European country. Nor can there be any question that another link with the capacity of the present channel ferries concentrated into the south-east tip of Britain would increase the congestion of the most crowded part of the country. There is considerable resentment already—in Belgium for instance—that so much freight has to be routed through France, picking up an additional and particularly swingeing tariff on the way. This has also put up the price of the goods in Britain.

There seems an excellent case for new links to the continent from the east coast of Britain and the more westerly of the south coast ports, thus better distributing traffic and avoiding the London bottleneck, and also bringing welcome growth to the less industrialised regions. This need not be motorised traffic either—whole trains travel by ferry in Scandinavia. The south coast port authorities have already indicated that they could handle twice the present traffic, which corresponds closely with the amount predicted for the tunnel at its planned opening date in 1981.

The potential of the hovercraft link seems to have been ignored in the predictions and calculations. Yet this type of ferry (using the current SRN-4, a 350-ton vehicle) takes only 30 minutes to cross the same route as the pro-

jected Chunnel, the journey in which was expected to last nearer an hour. Five craft now carry 25% of all passenger traffic on this route. Docking requirements, and therefore land use, is minimal. Apart from their rapidity, they perhaps represent the most flexible type of sea link yet devised. It is paradoxical that while the French have felt obliged to cry "perfidious Albion" again over Crosland's tunnel decision the partly state-subsidised Bertin company is hurrying to complete a larger cross-channel hover-ferry, the Naviplane 500, which is expected to be at the trials stage in 1976 and no doubt in competition with the British service shortly after. That there are no plans to develop a larger version of the SRN-4, nor a successor, is a matter of major concern in the industry.

Which brings one back to the lack of a true overall plan for traffic and transport development in Britain. What happened to the comprehensive independent study commissioned by the Ministry of Technology in the mid-1960s?

There is no reason to doubt that modern technology could build a channel tunnel if the nation was prepared to spend enough on it. If a prestige gesture for a technologically advanced (and united) Europe is a worthy object, then a Wilson-D'Estaing bridge would be much more telling than a Macmillan-De Gaulle tube. If better communications and a good return for money is the point, then the Secretary of State for the Environment has done the right thing and not a moment too soon, though it will be important to follow up a sensible saving with a sensitive alternative solution or solutions. The one bit of advanced technology that one must regret will not now come to fruition is the project of certain citizens of Kent for a Great Channel Tunnel Cork—to plug the great channel tunnel's mouth when it was finished. □

correspondence

Neuberger on nutrition

SIR,—I have considered myself a nutritionist for nearly 40 years, and I doubt whether even in the 1930s I would have ignored the teachings of John Boyd Orr and Frederick Gowland Hopkins in order to accept the description of nutrition that forms the first sentence of the Neuberger report: "The science of human nutrition is mainly concerned with defining the optimum amounts of the constituents of food necessary to achieve or maintain health."

Nutrition, on the contrary, is to do with the whole relationship between man and what he eats, that is, it is to do with food: how food is produced; what determines which foods we eat and how much; what the constituents of food are; which of these the body requires and in what amounts; how these constituents are dealt with in the body and what functions they perform; what happens when the required amounts of the required constituents are not provided or are exceeded; what steps can be taken to avoid these differences between what is needed and what is consumed. Nutrition, therefore, has reference to economics, anthropology, sociology, demography and psychology, as well as to chemistry, biology, biochemistry and physiology. In particular, nutrition is the one science that can least afford to remain in the laboratory; it concerns every single human being, every single day of his life.

What enthusiasm the report shows is confined largely to its summary of existing knowledge of the biochemical aspects of nutrition, and much of its recommendations for further research are also in this area. There is only an occasional brief reference to nutrition as it affects the whole body and an even less frequent nod towards the need to know more about people's nutritional behaviour. This bias is really not good enough; the human body exists together with other human bodies in a social and cultural environment, and important as biochemistry is, it is as important—and possibly more so—to know what determines the diets of different people, in different groups, at different times. Certainly we need to know more about energy transformation in the body and the mechanisms that control body weight and body composition. But of more immediate relevance to problems of malnutrition is the search for answers to quite dif-

ferent questions: Why do some people find it easy to cure their obesity and others find it difficult? Why are so many of the obese so easily persuaded that they can solve their problem by eating Ryvita or yoghurt, or by swallowing slimming pills that contain nothing but aperients, or by going to expensive but quite ineffective slimming clinics?

In this country, and in other countries in the western world, there is a considerable and increasing demand for so-called health foods. What is it that makes so many people entirely ignore the knowledge so laboriously acquired by nutritional research in favour of the incorrect or misleading information that makes them buy brown sugar, brown bread, sea salt, honey and vitamin pills to ensure that they are adequately nourished, or that makes them believe that there is special virtue in brown eggs or in vegetables grown with compost rather than with chemical fertilisers? And why do we continue to act as if it is still true that adequate nutrition in the industrialised countries is largely a matter of economic circumstance—as if all that matters is that a family shall have an adequate income—when it has been demonstrated in the USA, for example, that there has been an increase in the proportion of families eating a poor diet at the same time as family incomes have increased? Again it is just not good enough for the nutritionist to ignore these views, held as they often are by sincere and highly intelligent people, however misguided. Even less is it good enough for us to smile a superior smile and tell such people—and tell each other with a wink—how stupid these views are.

It is because nutritionists know that such matters are important that research carried out in departments of nutrition today is concerned not only with the physiological and biochemical problems that the report concentrates on so heavily, but with broader subjects too: the factors that determine food choice, the influence of diet on behaviour and of behaviour on diet, the assessment of attitudes towards foods and nutrition, and the differences between what people think about food and what in fact they eat. In the university department that I know—that at Queen Elizabeth College, London—more than 300 research papers were published between the start of the degree course in nutrition in 1953 and my retirement from the Chair of

Nutrition in 1971.

The report deplores the "relative neglect of human nutrition" as a subject for research. I suggest that this neglect is, more than anything else, a consequence of the existence of a myth about what nutrition is. The myth is that nutritional science is mostly to do with "defining the optimum amounts of the constituents of food". It is the narrowness of this account of nutrition that has been largely responsible for the subject's failure to attract either the research support or the attention of young scientists to the extent that its interest and importance deserve. Although the report deplores these consequences, it perpetuates the myth that has helped to create them.

Yours faithfully,

JOHN YUDKIN

London NW3, UK

Asbestosis

SIR,—The best comment to be made on P. F. Holt's letter (January 10) about complacency over asbestosis is contained in the latest Annual Report of HM Chief Inspector of Factories, which says in relation to new cases of asbestosis and mesothelioma, and the latest figures, that they "reflect conditions in the past when, in the then state of knowledge, it could not be ascertained with any certainty what levels of air contamination by asbestos dust would endanger health". Later, describing a major long term medical environmental survey of asbestos workers, he says: "In Phase II of the survey, which embraced the larger manufacturers of asbestos products, 5,000 workers were medically examined and 700 representative personal samples were taken in the workers' breathing zones over 4-hour working periods. The results, which were most encouraging, showed that 92.6% of the dust counts taken were below the very stringent hygiene standard of two fibres/ml and reflect the very great efforts made by the major firms in the industry to improve standards of control."

Mr Holt's references to a London life of insulation from reality are wide of the mark. I work in Manchester, the geographical centre of the British asbestos industry, and spend much of my time in asbestos factories.

Yours faithfully,

W. P. HOWARD

The Asbestos Information Committee,
Manchester, UK

Manpower needs

SIR,—We read with interest your editorial (January 3) entitled "Manpower needs are not so easily estimated". We feel that, although you made some telling points, the bluntness of your critical edge and the scathing tone caused some important aspects of manpower problems and the role of the Manpower Services Commission (MSC) to be lost. The baby, to our dismay, went out with the bathwater.

First of all, the nature of the MSC and its relationship to the Department of Employment are by now well known to those with a practical interest in the field and are easily obtained. Perhaps the opportunity to publicise its role might have been taken by a note on the fly leaf of the report on the manpower implications of offshore oil and gas. It is a pity, then, that you got your facts wrong and referred to both the MSC and the Petroleum Industry Training Board as agencies of the Department of Employment. And in drawing attention to a misprint you tempted providence in the form of the printer's devil, who inserted another in the same line of your own piece.

So, please let all of us eliminate, as you say, the "emotive turn of phrase" and give some careful thought to the critical problems of manpower—of which the manpower to enable us to exploit offshore oil and gas is not the least.

Perhaps the wisest statement of your editorial was the title: manpower needs are not so easily estimated. The UK has devoted ludicrously small resources to studying and forming policy about manpower. It is, after all, perhaps our most critical national resource (and that statement is fast becoming a cliché) and yet, as you rightly point out, we have not had the major, in depth, study that is really necessary to provide a sound basis for action at all levels in respect of skills and manpower needed to exploit the North Sea.

In such a situation, the arrival of the MSC as an independent voice is an important step forward. Its role and composition was established by the Employment and Training Act 1973. The MSC is financed through the Secretary of State for Employment (but is not part of the Department) and through the Training Services Agency co-ordinates the operation of the Industrial Training Boards.

At this early stage, it is surely appropriate for the MSC to promote and publicise pieces of work, particularly those that summarise what is known. In the process, some ideas will come forward to be dismissed and some good work will be overlooked. All this will draw attention to the paucity of current knowledge.

The report that you criticise, to quote the introduction, attempts to provide an overview by drawing together the threads of previous studies. There is no guarantee that a literature search will provide the answers to any problem. In the circumstances, and in view of the public debate that is taking place, the MSC is surely right to publicise the findings, but not to endorse necessarily the conclusions reached. In that way we will all learn.

Perhaps we could also take the opportunity to make some comment on the problems of the supply of qualified manpower, drawing on some of our own research. By and large, the employing organisations which create demand for people, on the one hand, and the education and training institutions which create a supply, on the other, operate pretty well independently of each other. Any impression that we can forecast the demand for (for example) geologists and then set up the courses to provide them is so oversimplified as to be useless. Accurate forecasts are not possible in any case. We have to know more about the absorption of graduates into the economy—some emigrate, others go back into education, others move rapidly away from straight application of their basic discipline. More needs to be learned about how university courses might develop in the interests of the individual and society (taking a very long term view). Even if we knew precisely what we wanted, there remains the question of how the educational system and the people in it react or might be helped to react. Industry is notoriously weak at articulating a considered view (though you are right to say that many companies prefer to undertake specialist training of geologists themselves).

Thus, in regard to many aspects of manpower, we have an extraordinarily complex system working within a changing and unpredictable environment in which the components have very strong needs of their own.

Manpower needs are indeed not so easy to estimate. We trust we may see more encouragement of and support for those who are only now beginning to grapple with the problem.

Yours faithfully,

JOHN LAWRENCE
GRAHAM ATKINSON

*Institute of Manpower Studies,
London School of Economics*

International meetings

SIR,—The letter from S. Peller (December 13) is similar to so many of the past few years and raises again the recurring, vexing problem of the location of international conferences. Clearly

assurances by some governments of entry to all participants are meant by these governments to be honoured in the breach. Starting from the premises that international scientific meetings should, in fact, be open to all qualified scientists, and that the internal affairs of the host country should not be interfered with, I should like to make the following suggestion.

Each international union should set up a committee to investigate complaints from *bona fide* scientists who have been barred from a meeting. If the charges are verified, then the union would bar that country as host for other meetings for a period of five years, the restriction to apply even to meetings already scheduled. Thus any country would have the choice of playing politics or hosting international meetings, but not both. Of course, some measures would also have to be taken to prevent the scientific union itself from becoming political, as has happened to UNESCO.

Variants of this method could also be considered. Thus a country which does not allow its scientists free access to meetings, such as, for example, the Soviet Union, could be barred from having any of its scientists attend such meetings.

Yours faithfully,

SAM SILVERMAN

Lexington, Massachusetts 02173

Alien message

SIR,—If an alien civilisation should chance to receive the radio message broadcast to the stars from the Arecibo telescope (January 24) it would be fully justified in reversing the charge for any reply. I make this assertion because, to judge from your illustration, the original communication was itself reversed, vertically. Which is to say, sir, it was upside down (not that the concept of tops and bottoms means very much in terms of inter-galactic messages).

As the message stands, an alien listener might just about make out that we are trying to tell him something about a man who appears to be balancing on his head at the top of a Maypole.

I for one shall be surprised if anybody (from this galaxy or the next) troubles to reply to such blather, unless it is to tell us that his chaps go in for Morris dancing while walking on their hands.

Yours faithfully,

B. JOVE

Hove, UK

ED—Sorry; the message was garbled during transmission from the *Nature* office to the printing works. So much for the march of progress.

news and views

New nitrogen-fixing symbioses *in vitro*

from John Postgate

THE truism that rhizobia, the symbiotic nitrogen-fixing bacteria, never fix nitrogen away from a leguminous host plant may seem, to an outsider, more of a dogma than an experimentally based fact. But a convention of scientific publication is that one rarely publishes purely negative findings, so the absence of documentation in the scientific literature of this failure does not do justice to the effort which has been put into the question: the truth is that many workers throughout the world have attempted, without success, to obtain fixation by pure cultures of rhizobium *in vitro* using all sorts of plausible conditions, such as nitrogen starvation at low oxygen tensions, presence of plant extracts, glutamine mutants and so on. A report in 1971 (*Nature*, **232**, 173) in which research workers from the laboratories of Dupont de Nemours obtained nitrogen fixation in a tissue culture of callus from a soyabean root, infected with *Rhizobium japonicum*, was greeted with excitement as a step in the direction of simplifying this nitrogen-fixing association. Fixation, though slight, was apparently taking place without the nodulation, leghaemoglobin and bacteroid formation seemingly necessary in the intact plant.

Though confirmatory reports gradually appeared from other laboratories, some scientists remained sceptical. Ethylene formation from acetylene, a diagnostic reaction for the nitrogen fixation enzymes, was the criterion used in these experiments; but ethylene is a normal product of plant metabolism. Were the controls for endogenous ethy-

lene as reliable as they looked? In addition, persons working with such material know well that it is very difficult to rid callus cultures of bacteria completely and the contaminants which persist are often capable of nitrogen fixation alone. They also knew that it is difficult (because of the gummy nature of the colonies) to be wholly certain that cultures of rhizobium are pure. Finally, the critics knew that aerobic organisms—bacteria or plant cells—could scavenge oxygen from a micro-environment and enable anaerobes such as *Clostridium pasteurianum* to grow and fix nitrogen. Were the microbiological controls of the early experiments adequate? Certainly they were cursory as described in the earliest publications and sometimes even absent.

Considerations of this kind account for the admirable preoccupation with cultural purity noticeable in two important developments, reported simultaneously from Canada and Australia in this issue (pages 350 and 351). Both papers present evidence that rhizobia, at least of the slow-growing 'cowpea' type, can form associations *in vitro*, not only with callus from the leguminous plants with which they associate in nature, but also with callus from plants which do not normally nodulate and even with callus from quite unrelated plant groups.

Like the Dupont group, all the workers obtained their rhizobia from Dr Burton of Milwaukee. Child not only checked for contaminants carefully but used three different strains of rhizobium and callus from six different types of plant, thus diminishing the

possibility that he is handling artifacts of contamination; Scowcroft and Gibson have also checked their material microbiologically and, in addition, with isotopic $^{15}\text{N}_2$, thus completely disposing of any possibility of errors due to endogenous ethylene production by the plant material. So, accepting that their meticulous negative tests for contaminating nitrogen-fixing microbes were adequate, the reality of effective nitrogen-fixing associations between slow-growing rhizobia and plants as remote from legumes as tobacco or the monocotyledonous wheat seems established, at least *in vitro*. It is entirely consistent with these findings, as both papers acknowledge, that Trinic recently discovered a natural nitrogen-fixing association between a slow-growing rhizobium and a non-leguminous plant of the genus *Trema* (*Nature*, **244**, 459; 1971). But the new experiments go further than this: their design is such that the plant callus is grown on a membrane, and this lies on an agar surface which has been infected with rhizobia. By removing the membrane the plant material can be removed from at least a proportion of its associated rhizobia. Yet the rhizobia remaining after the plant material had been removed continued to fix nitrogen for several hours. For the first time, therefore, rhizobia have been induced to fix nitrogen away from the plant, though the ability does not persist on subculture.

Physiological studies made it clear several years ago that rhizobia are the sites of nitrogen fixation in the nodule. Whether or not they possess the genetic information necessary to form the enzyme system is still not certain, but it is clear that, to use their information, they need something from the plant. The new experiments suggest that the material needed is diffusible and that its effect lasts for several hours. In the short term, biochemists, plant physiologists and geneticists will await eagerly the characterisation of the diffusible factor and the understanding of its apparent role in the regulation of the nitrogen fixation genes of rhizobium. In the longer term, these studies are a useful step towards the establishment of nitrogen-fixing associations between rhizobia and all sorts of agriculturally important crops and forage plants.

Elm bark disease in Australia

from Allan Rosel and John French

THE smaller European elm bark beetle (*Scolytus multistriatus*) was discovered in 1974 to be attacking elms in and up to 123 miles from Melbourne. This beetle is one of the main carriers of the Dutch elm disease fungus (*Ceratocystis ulmi*). It has not previously been recorded in Australia although the number of emergence holes in the bark of infested elms suggests that the beetle has been established in Victoria for several years. The beetle has been

sought but as yet not found elsewhere in Australia. A survey is continuing.

Microbial examination of beetles and attacked trees has failed to reveal the presence of the Dutch elm disease fungus. Dr S. L. Wood of Brigham Young University, Utah believes that the disease, if present, would have been apparent by now. It seems that in Australia, as on the west coast of North America, the carrier is present but not the disease.

New steps in the formation of phage T4 heads and DNA packaging

from P. J. G. Butler

THE large bacteriophage T4 has a long history of interest and current developments are maintaining this standard. Since it was shown to have a DNA-containing head connected by a hollow tail to the fibres which are involved in the attachment to the host cell, interest has centred on the mechanism for the assembly of such a complex structure. As shown by the groups at Caltech and Geneva, it is particularly suitable for such study because of the ability of the incomplete fragments, which accumulate in cells infected with mutants in many of the assembly steps, to form complete phage either by *in vitro* complementation with the fragments from another suitable mutant (Edgar and Wood, *Proc. natn. Acad. Sci. U.S.A.*, **55**, 498; 1966) or by subsequent activation of the blocked step (for example by the removal of a temperature sensitive infection from a non-permissive temperature to a permissive one).

The assembly occurs as a series of subassembly steps, with the head, tail and tail fibres being assembled separately and then joined together, first the tail on to the DNA-containing head and finally the tail fibres added to the fibreless particle to give the complete phage (reviewed by Wood *et al.*, *Fedn. Proc.*, **27**, 1160; 1968). During the infection of a host cell, the incoming DNA is expelled from the head through the tubular tail without any requirement for an energy source. This has led to speculation that the DNA is packed into the head in a special way, as if under pressure, so that its release occurs like that of a spring. A highly condensed state for the DNA is also required for the total amount to be packed into the volume available.

Head assembly

Unlike a number of simpler viruses, the heads of T4 seem to be largely assembled before much of the DNA is inserted into the pre-existing shell (Luftig *et al.*, *J. molec. Biol.*, **57**, 555; 1971; Simon, *Proc. natn. Acad. Sci. U.S.A.*, **69**, 907; 1972), and recently some of the steps have been described. A hierarchy of partially characterised steps (Laemmli *et al.*, *J. molec. Biol.*, **49**, 99; 1970) leads to the formation of the first recognisable head structure, prohead I, with a sedimentation coefficient of about 400S, containing at least four protein species (P23, P20, P22 and IP III) but no DNA (Laemmli and Favre, *J. molec. Biol.*, **80**, 575; 1973). These particles are converted to prohead II (about 350S) by the cleavage of

the major protein (P23) to give P23*. This cleavage seems to require the action of two distinct phage proteins, P21 and P24, and failure of either of these leads to the accumulation of 'τ-particles' (Epstein *et al.*, *Cold Spring Harb. Symp. quant. Biol.*, **28**, 375; 1963; Laemmli *et al.*, *J. molec. Biol.*, **49**, 99; 1970) which are smaller than the normal heads.

Though the τ-particles produced in the absence of functional P21 seem to be completely aberrant and cannot be rescued to form normal heads (Laemmli and Johnson, *J. molec. Biol.*, **80**, 601; 1974), those formed without P24 can act as a subassembly for head formation, without being disrupted in the process (Bijlenga *et al.*, *Nature*, **249**, 825; 1974). Analysis of the proteins in these 24 τ-particles shows that they

contain uncleaved P23 and that this is converted to P23* upon activation of P24, and it thus seems probable that these τ-particles represent an intermediate in the pathway between proheads I and II (Bijlenga *et al.*, *J. supra-molec. Struct.*, **2**, 45; 1974).

The conversion of prohead II into prohead III (550S) again involves the action of two phage proteins, P16 and P17, and is the first step in which the proheads become associated with the replicative DNA and the DNA packaging starts. During this conversion of II to III the cleavage of two further proteins, P22 and IP III, begins. This process is finished during the final maturation of the prohead III into mature phage heads. This requires the action of yet another protein, P49, and goes in close parallel with the com-

The magnetic field of Jupiter

from D. Stannard

THE latest development in the exploration of the Solar System by spacecraft came with the arrival of the Pioneer 11 probe at Jupiter in early December. Following almost a year after Pioneer 10, its predecessor to the planet, Pioneer 11 swept within 0.6 planetary radii (R_J) of the Jovian surface before continuing on its way towards the planned encounter with Saturn in 1979. With remarkable promptness, Acuna and Ness present a preliminary interpretation of the Pioneer 11 measurements of the structure of the inner Jovian magnetic field in this issue of *Nature* (page 327).

Ground-based studies of the radio emission from Jupiter over the past twenty years have suggested a picture of the field structure which is basically that of a magnetic dipole, with the axis of the dipole inclined by just under 10° to the rotation axis of the planet. Asymmetries which are present in both the decametric (noise burst) and decimetric (synchrotron) radio emission suggest that there may well be fine structure associated with the dipole field at distances of between 1 and 2 R_J from the planet. In particular there is evidence of a localised field distortion close to the longitude of the magnetic pole in the Northern Hemisphere (Conway and Stannard, *Nature*, **239**, 142; 1972).

The magnetometer measurements from Pioneer 10 confirmed the essential dipole character of the main Jovian field at distances greater than the spacecraft's closest approach of 2.8 R_J . A better fit to the data in the inner field region was however obtained using a 'dual dipole' model, with a weak secondary dipole close to the position of the field anomaly indicated by the radio astronomy measurements (Smith *et al.*, *J. geophys. Res.*, **74**, 3501; 1974).

The close approach of Pioneer 11 to the Jovian surface has now enabled the structure of the inner field region to be studied in detail. As shown in Figure 3 of Acuna and Ness's paper, the field is indeed complex, and at least an octupole expansion is required to describe the data. Particularly prominent is the asymmetry in the pole strengths of the two hemispheres, with a field of 28 Gauss at the north magnetic pole, and only 16 Gauss at the south. The magnetic equator within 2 R_J is highly distorted and steeply inclined with respect to the zenographic equator. With this detailed information about the field structure it should now become possible to perform more reliable quantitative calculations on the radio astronomical data to enhance our knowledge of the physical processes in the Jovian magnetosphere.

pletion of the DNA packaging. While a large part of the cleaved IP III remains in the mature heads, as IP III*, most of the cleaved P22 is lost and only the 'internal peptides' remain in the mature heads (Showe and Black, *Nature new Biol.*, **242**, 70; 1973).

Laemmli and Favre had shown that prohead II contained less than 1% of the DNA content of full heads and obtained some evidence that the prohead III contained about 50% of the DNA. This estimate has now been confirmed by the incorporation of bromodeoxyuridine into the maturing prohead III particles when a temperature-sensitive P49 is activated by a temperature shift, with further protein synthesis inhibited (Laemmli *et al.*, *J. molec. Biol.*, **88**, 749; 1974). After isolation of the DNA from the mature phage it was cleaved at the sites of bromodeoxyuridine incorporation, by exposure to ultraviolet light, and then found to have been reduced to about half its intact molecular weight. Using a similar technique, these authors also confirmed that the 24 τ -particles (and, by presumption, prohead I) contained little DNA.

The problem of the length determination for the DNA packaged into a mature phage particle has been further investigated using the giant heads produced by exposure of infected cells to L-canavanine and then deinhibition of the DNA synthesis with arginine. After isolation of narrow length classes, of about 4x and 8x normal head lengths from T4 and 12x to 13x from T2, and very gentle lysis of the phage to give intact DNA, it has proved possible to measure the molecular weight of the largest DNA molecules in the giant heads (Uhlenhopp *et al.*, *J. molec. Biol.*, **89**, 689; 1974). Using the technique of viscoelastic relaxation, these authors determined not only the molecular weight of these large DNA molecules (up to 1.5×10^9 daltons) but also the fraction of the total DNA which they represent. Though the large molecular weights found were fully consistent with the hypothesis that the DNA is packaged and only cleaved once the preformed head is full, without the need for any other size determining mechanism, the presence of about 95% of the DNA in the largest heads in smaller pieces means that the evidence is not conclusive against some additional mechanism.

Host cell membrane

Another interesting area of the interaction of T4 with its host cell is the involvement of the host cell membrane in the assembly of the phage heads. It has been known for some time that the head proteins are associated with this membrane and that this association is mediated through the phage protein P31 (Laemmli *et al.*, *J. molec. Biol.*, **47**,

69; 1970), but also mutant *E. coli* have been isolated which although permitting phage absorption will not support virus production (reviewed by Wood *et al.*, *1st John Innes Symp.*, 25; 1972). In one particularly interesting 'host defective' mutant cell line it has been found that the phage heads made are apparently normal, yet they are not filled with DNA and the rate of phage DNA synthesis is abnormally low (Simon *et al.*, *Nature*, **252**, 451; 1974). Two phage mutants have been isolated, which are able to overcome this block, and one of them is again in protein P31. Further evidence that the blocking may be due to alteration of the host cell membrane comes from the observation that it could be overcome by very low concentrations of chelating agent, which have their primary effect on the

bacterial membrane.

These results suggest that the host cell membrane plays a major part in the phage DNA synthesis. It also seems to have another, and rather less obvious, function during the disruption of the host cell DNA which follows infection by T4. This occurs by a dispersal of the host nucleoid with the association of the DNA with the cell membrane and its subsequent hydrolysis. Two 'nuclear-disruption deficient' phage mutants have now been isolated, which fail to cause this association although the host DNA does still slowly disperse throughout the cell and is hydrolysed normally (Snustad and Conroy, *J. molec. Biol.*, **89**, 663; 1974; Snustad *et al.*, *J. molec. Biol.*, **89**, 675; 1974). After comparing the DNA hydrolysis and release in various endonuclease-deficient mutants

Reversals through time

from D. H. Tarling

SEVERAL recent letters to *Nature* show increasing interest in the possible causal relationship between changes in climate and changes in the intensity of the Earth's magnetic field. Such correlations, if substantiated, would explain the extinction of various marine microorganisms at times of geomagnetic polarity reversal as, at such times, the Earth's field drops to about one-fifth of its usual value. The occurrence of polarity transitions during geological time is therefore of biological and climatological interest as well as being critical to models for the generation and evolution of the geomagnetic field.

The difficulty in studying such polarity reversals is that they are brief, lasting for some 10^3 to 10^4 years, in contrast to constant polarity periods which persist for up to 50 million years. This brevity makes them difficult to study as sedimentation in the deep oceans is too slow and lava eruption is too spasmodic to permit detailed palaeomagnetic studies, except in rare situations such as where the changing magnetic vectors have been trapped during the slow cooling of a large intrusive body. Even so, the average features of reversals during the last 70 million years are fairly well known and the pioneer studies of van Zijl *et al.* showed that their properties are similar to those of a reversal some 160 million years old in South Africa (*Geophys. J. R. astr. Soc.*, **7**, 169-182, 1962).

Some 2,000 years before a change in direction, the geomagnetic field intensity decreases by 80%, after

which the direction takes about 3,000 years to reverse and is followed by 2,000 years during which the intensity returns to its usual value. It is unlikely that the time scales of individual transitions differ from such generalised figures by more than an order of magnitude and the sequence seems to be consistent. Similar changes with similar time scales also occur during geomagnetic 'events' when the geomagnetic pole seems to topple, but then returns to its previous polarity without a true reversal having occurred.

These short time scales indicate that reversals must result from short-lived geophysical processes at the core-mantle interface. The identification of generally similar features during a polarity reversal some 1,600 million years ago, as described by Bingham and Evans on page 332 of this issue, is critical to geomagnetic climatic and evolutionary models. But it also has implications for the evolution of the interior of the Earth as a whole. Since essentially identical processes seem to have been involved at the core-mantle interface in mid-Precambrian times the basic parameters of the Earth, such as the dimensions and composition of the core and lower mantle, cannot have changed significantly since then. Hence it seems unlikely that any substantial differentiation can have taken place within the mantle within the last 2,700 million years (since Archean times), and convection in the mantle since then would, therefore, have to have been shallow.

of both the wild type and the nuclear-disruption deficient phage, these authors put forward a model for the nuclear disruption process. They suggest that the host DNA becomes attached to the cell membrane at several hundred sites during a normal infection. In an uninfected cell it might be attached at about five sites—the origin and terminus of replication and a few replication forks—while in the nuclear-disruption deficient infected cells it is probably attached at fewer than ten sites. Surprisingly, in spite of this major change in the effect of the phage on the host cell DNA, no difference was detected in either the burst size or the growth rate of the mutants and the wild type.

These various results show that the growth and assembly of T4 is still a subject of considerable interest and give hope that some of the intriguing problems may be cleared up soon.

Poly(A) tales

from Tim Hunt

THE November issue of the *Proceedings of the Academy of Sciences of the USA* contains an elegant demonstration that the poly(A) tail of globin mRNA is unnecessary for its translation in cell-free protein synthesising systems. Sippel *et al.* (Columbia) (71, 4635; 1974) hybridised globin mRNA with oligo(dT) and then attacked the hybrid with RNase H, a nuclease that specifically digests the RNA strand of a DNA-RNA hybrid. After this treatment, the reaction mixture was passed over a mixed gel filtration and nitrocellulose column to remove the oligo(dT) and undigested hybrids. The mRNA that resulted was shown to be free of poly(A) by its failure to form dsRNA with ³H poly(U).

It would be hard for these authors to be sure that they had removed literally all the poly(A); the possibility that a few residues remain would not, I think, be excluded. Nevertheless, this method for removing the poly(A) has advantages over the use of polynucleotide phosphorylase or the 3' exonuclease from eukaryotic nuclei. These exonucleases are processive enzymes—they do not leave their substrate until it is completely digested; hence, in order to digest all the molecules in a message preparation a molar excess of enzyme must be added, which in turn means that it must be highly pure. In addition, it is hard to stop the enzyme from going past the poly(A) stretch into the structural mRNA. Lastly, the RNase H method has a universal application for the removal of specific pieces of RNA if the complementary piece of DNA is available.

So what happens when you remove poly(A) from mRNA? The answer is generally agreed to be almost nothing. As a template for globin synthesis in cell-free systems (wheat germ, Krebs II ascites extracts, Schreier and Staehelin) the deadenylated mRNA is just as active as its unassaulted parent. This conclusion has been reached before by Williamson *et al.* (*Biochemistry*, 13, 703; 1974), Bard *et al.* (*Cell*, 1, 101; 1974) and Soreq *et al.* (*J. molec. Biol.*, 88, 233; 1974), all of whom used processive exonucleases to remove the poly(A); Williamson *et al.* and Bard *et al.* clearly did some damage to the mRNA beyond removal of the poly(A), but Soreq *et al.* used highly purified enzyme under very restrictive conditions, and their data are extremely similar to those of Sippel *et al.*

They did notice that the activity of the deadenylated mRNA tailed off earlier than that of the native mRNA, although the effect is not dramatic. Sippel's group also report a lowered stability *in vitro*, but the cell-free system itself loses a lot of activity over the same period of time, and the decay of the message has curious linear kinetics which is unusual; normally mRNA decays exponentially. In any case, it may be dangerous to argue from cell-free experiments to the situation within living cells, and with this caution in mind, Soreq's group arranged for their mRNA to be injected into *Xenopus* oocytes (Huez *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, 71, 3143; 1974). They found that both the activity and the stability of the deadenylated mRNA were apparently impaired. I think there are problems in the interpretation of these kinds of experiment, although the experimental data are impressive. The trouble lies in knowing how to quantitate the amount of protein formed after injection of mRNA, owing to uncertainties about the degree of penetration of label and its dilution with endogenous amino acids. Huez *et al.* take as their measure a comparison between radioactivity in the 'endogenous' proteins and that in the peak of globin from a gel filtration column. The tacit assumption that the rate of endogenous protein synthesis is constant is very hard to test, and may not be correct.

Interesting and pertinent studies of the stability of RNA after its injection into oocytes have been made by Allende, Allende and Firtel (*Cell*, 2, 189; 1974). They injected radioactive RNA and followed the fate of the label rather than the stability of the template activity of the mRNA. They found that the RNA was degraded at an appreciable rate, except in the cases of tRNA and poly(A) itself; however, the decay of mRNA followed biphasic kinetics. This apparently results from the interaction of mRNA with cellular com-

ponents, possibly ribosomes, since the stable fraction is not seen in the presence of puromycin. These are provocative findings in the light of the extreme stability of the translational capacity of injected globin mRNA (Gurdon, Lingrel and Marbaix, *J. molec. Biol.*, 89, 539; 1973) and also of the fact that oocytes contain respectable amounts of stored mRNA which survive from oogenesis to fertilisation (Adamson and Woodland, *J. molec. Biol.*, 88, 263; 1974). The mechanisms ensuring on the one hand the stability of these messages, and on the other their unmasking during development are quite obscure. There were certainly no indications in Allende *et al.*'s experiments that the poly(A) played a part in the stabilisation of the injected messages; it would be interesting to repeat these experiments with radioactive globin mRNA with and without its poly(A), not an easy thing to arrange except by iodine labelling, since very high specific activities are necessary.

Taken together, these experiments suggest that removing the poly(A) may reduce the stability of the mRNA in certain circumstances; but there is little evidence from studies on intact cells to support the hypothesis that a long stretch of poly(A) confers a long life on a message. Although the poly(A) gets shorter with age (Sheiness and Darnell, *Nature new Biol.*, 241, 265; 1973), it seems that old messages have a similar life expectancy to young ones in view of the well established exponential decay of mRNA (see for example Brandhorst and McConkey, *J. molec. Biol.*, 85, 451; 1974). The recent description of mRNA which lacks poly(A) in HeLa cells (Milcarek, Singer and Penman, *Cell*, 3, 1; 1974) includes data on its lifespan, and there are no indications of anything unusual about the non-adenylated species compared with the adenylated. The poly(A) of mRNA still seems to be more of a natural gift to the molecular biologists than an essential component of mRNA. Yet the refrain is always: 'It's there, so it must do something'.

Histamine and the brain

from a Correspondent

At a meeting of the Collegium Internationale Neuropsychopharmacologicum in Paris last July, ideas about the meaning of histamine in the brain were brought together and aired (*J. Pharmac. (Paris)*, 5, Supp. 1, 69; 1974). From this airing, it emerged that histamine is present in the brain, although in smaller amounts than are its relatives, 5-hydroxytryptamine and noradrena-

line. The histamine is partly in mast cells, but partly also in nerve terminals. It is distributed unequally in the various regions of the brain, the region richest in histamine being the hypothalamus, within which different nuclei have widely different contents. The l-histidine decarboxylase that produces the neuronal histamine is also present and achieves a rapid rate of histamine synthesis, naturally followed by rapid breakdown. In experiments in which mechanical lesions were made in the medial forebrain bundle (an area associated with reward), a decrease of the histamine content and of its synthesising enzyme suggested that a tract of histaminergic fibres had been severed. All this evidence, much of which can also be found in a well-documented paper by M. Garbard and colleagues (*Science*, **186**, 833; 1974), encourages the belief that histamine should be added to the list of putative mediator or modulator substances involved in brain function.

One of the best ways of deciding whether a putative mediator is a really serious candidate is to study the effects of its specific antagonists. Such tests, with the classical anti-histamines, whose development was started by Daniel Bovet and colleagues before the second World War, have clearly indicated that histamine plays a part in anaphylactic bronchoconstriction in the guinea pig and in nasal and dermal allergy in man. This criterion also suggested that histamine might be involved in such central nervous activities as wakefulness and motion sickness, because conventional anti-histamine drugs have sedative, hypnotic and anti-emetic properties.

These anti-histamines, however, failed in several directions in which they were at first expected to act; for example they did not inhibit gastric secretion induced by histamine. This difficulty led to the recognition of H_1 and other (later called H_2) receptors (Ash and Schild, *Br. J. Pharmac. Chemother.*, **27**, 427; 1966) and to the development of antagonists for the H_2 receptor and agonists for both types of receptor that were selective to the point of specificity (Black, Duncan, Durant, Ganellin and Parsons, *Nature*, **236**, 385; 1972).

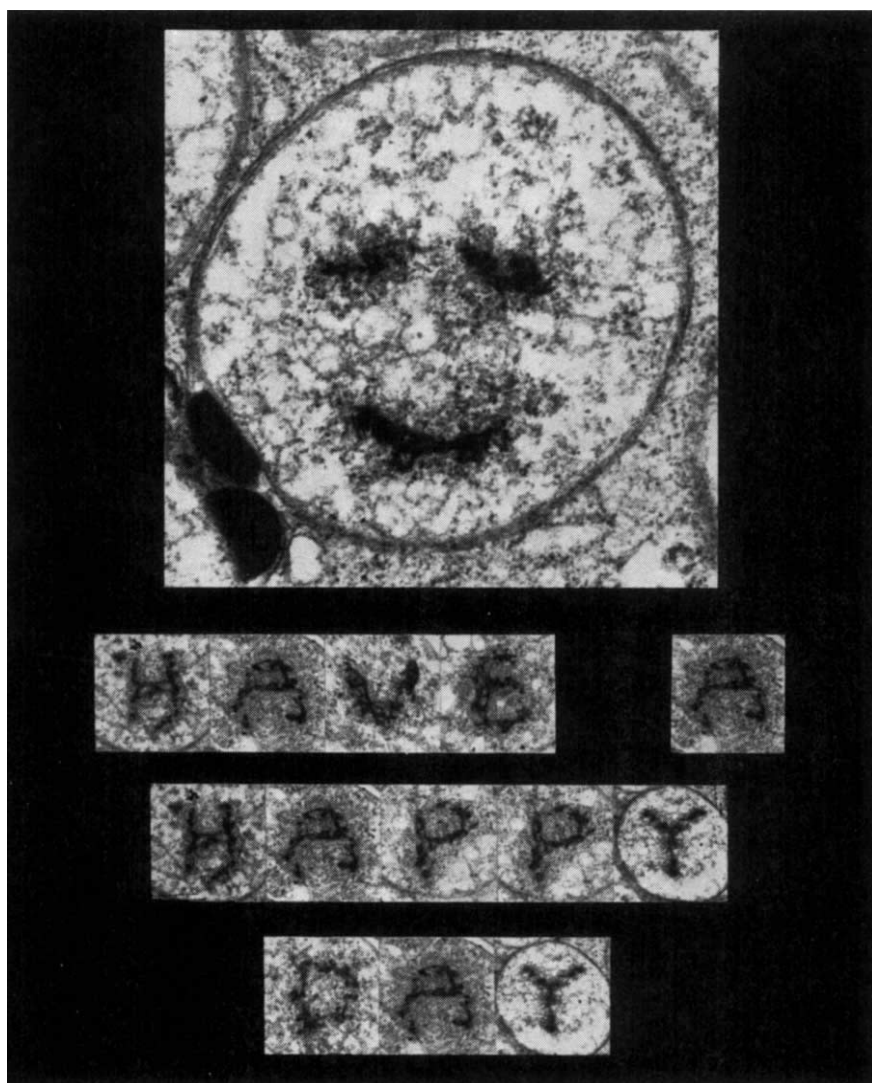
As so often happens, the availability of a specific receptor antagonist enables the action of the agonist to be clarified. In this issue of *Nature*, Baudry, Martres and Schwartz (page 362), report some insights gained from using specific H_1 and H_2 receptor antagonists (mepyramine and metiamide respectively) and an H_2 agonist (4-methylhistamine) to analyse the mechanism whereby histamine stimulates cyclic AMP formation in slices of guinea-pig cerebral cortex, an effect first observed

some years ago in rabbit cortex by Kakiuchi and Rall (*Molec. Pharmac.*, **4**, 379; 1968). The study of Baudry and colleagues shows that histamine acts on both H_1 and H_2 receptors to stimulate cyclic AMP formation, because it is not until both types of receptor are blocked that the effect of histamine is

obliterated. This finding fits with clinical experience, in that the central nervous effects of antihistamines of classical type are usually evident, but seldom intense. It would be interesting to know whether both H_1 and H_2 receptor blockers, given together, would produce profound sleep.

Cracking the genetic code is peanuts

from A. S. Craig and K. L. Giles



Genetic material of bacteroids in peanut root nodules, showing the only message de-coded to this date. Magnifications various.

DURING a study of the fine structure of root nodules from peanut (*Arachis hypogaea*) some features of considerable interest have been found. Routine electron microscope preparations of nodule tissue revealed plant cells full of large spherical bacteroids whose nuclear (genetic) material stained unusually heavily.

It did not pass our notice that the form of the genetic material in section often took on the appearance

of letters of the Roman alphabet. Armed with a knowledge of the genetic code one might have expected to find only the letters A, C, G and T. But so many were the letters found that it was possible to place several interpretations on them, one of which is shown. On these preliminary findings we are preparing a model of the genetic code based not so much on the idea of a macromolecular databank, as on a form of sophisticated semaphore.

The use of specific H_1 and H_2 antagonists to analyse brain histamine action has been carried also into another phylum. Recently, Nahorski, Rogers and Smith (*Br. J. Pharmac.*, **52**, 121P; 1974) in experiments carried out in the cerebral hemispheres of the chick, *in vivo* and *in vitro*, found that H_2 receptor antagonists blocked histamine-induced cyclic AMP formation, whereas an H_1 antagonist was ineffective. Thus, the chick may differ from the guinea pig in that histamine receptors on its cortical adenylyl cyclase may be largely of H_2 type, whereas in the guinea pig both types seem to be represented.

Glassy crystals

from Robert W. Cahn

In his stinging attack on Plato's political philosophy, Karl Popper (*The Open Society and Its Enemies*, chapter 3; Routledge, 1945) castigates Plato's "methodological essentialism", the delusion that to seek, by defining it, the essence of a thing can be a valid way of arriving at knowledge about the external world—including the world of social relations. Popper assures us that a true scientist always steers clear of questions which tempt him to definition-mongering, such as "What is an atom?" I am not at all sure that this is universally true: a passion for definitions sometimes provokes a scientist into a programme of experimentation which he might otherwise never have undertaken. The quest for the word provokes the deed.

These reflections are aroused by an unusual paper on the glassy state (Suga and Seki, *J. Non-Cryst. Solids*, **16**, 171; 1974). The authors, chemists at Osaka University, consider various ways of making non-crystalline solids other than by supercooling of a liquid, and ask whether it is plausible to call such solids "glasses". By itself, such a question deserves all the obloquy that Popper would direct upon it; but Suga and Seki went on to point out that the existence of a glass transition temperature is the crucial characteristic that has gradually come to be the defining criterion for glassiness. Thereupon they were moved to undertake a systematic study of glass transitions in a number of pure compounds nudged into non-crystalline states by cooling the melt, by condensing the vapour or by other more unorthodox means. In the course of these studies, performed in specially designed precision calorimeters (DTA apparatus), the authors discovered a number of anomalies: one compound might show more than one glass transition, and the concept of glassiness therefore required subdivision. It is rather as though St Thomas Aquinas

had conducted microscopic observations on the creatures cavorting on his needle-point and decided that it was necessary to distinguish archangels from mere angels.

Apart from a number of simple organic compounds such as isopentane, methanol, isopropylbenzene and cyclohexanol, the authors also examined a number of inorganic and organic high polymers, some inorganic materials (H_2O , $SnCl_2 \cdot 2H_2O$) and a range of compounds that form nematic or smectic (liquid crystal) phases. Thirty-eight materials were studied.

With regard to many of the simple compounds, it turned out that ordinary glass transitions, in the form of anomalies in the specific heat, turned up irrespective of whether the non-crystalline forms were prepared by supercooling a liquid, by condensation from the vapour or by rapid precipitation from solution. This answered the original question.

But three kinds of anomalous glass transitions were also found in some materials in addition to the ordinary transitions. Some compounds (for example, cyclohexanol, 2,3-dimethylbutane) have been known for some time to possess an intermediate structure at temperatures between full crystallinity and full liquidity, the so-called 'plastic crystal' state. The molecules are positionally ordered but orientationally disordered and mobile. This makes the molecules dynamically pseudo-spherical; because of this, they crystallise in face-centred cubic or body-centred cubic structures, which are unusually prone to plastic deformation: hence the name. Suga and Seki found that by rapid cooling they were able to freeze in this anomalous state, which they christened glassy crystal; on gradual reheating, the metastable glassy crystals passed through a 'glass transition' at which the metastable form transformed irreversibly into a fully ordered crystalline polymorph.

Another form of glassy crystal was found by supercooling of water, $SnCl_2 \cdot 2H_2O$ or $SnCl_2 \cdot 2D_2O$. Here the partial disorder was attributed to a freezing-in of the irregular positions of protons or deuterons; in this connection, the different transition temperatures for the last two compounds were of particular interest. There is a particularly full historical discussion of glassy ice—which, incidentally, has nothing whatever to do with the notorious polywater of a few years ago!

Finally, Suga and Seki examined a number of liquid crystals, in which there is orientational order, accompanied by positional disorder of molecules. It proved possible to 'freeze' this state by rapid cooling. The steady variation with temperature of the degree of molecular alignment, in the

'swarms' which are the liquid-crystal equivalents of ferromagnetic domains, is thereby inhibited. When the glassy liquid crystal, as the authors call this state, is heated through a transition, the molecules regain their labile ability to vary their degree of alignment.

Two compounds—cyclohexene and ethanol—were found to possess two anomalous transitions each as well as a normal transition. The nature of the two anomalous glassy crystalline states is not yet known.

The authors document their findings not only by locating glass transitions but also by computing the variation of entropy with temperature: from these plots they are able to make further deductions about the nature of the change they have observed.

The concept of a glassy crystal was sometime a paradox, but now the time gives it proof. Suga and Seki's paper is an impressive achievement which should stimulate many structural studies to confirm and extend their hold but sometimes tentative interpretations. Such studies may also have a bearing on topochemical reactions (see the detailed and very instructive review by J. M. Thomas, *Phil. Trans. R. Soc.*, **277**, 251; 1974). Topochemistry is concerned with the nature and kinetics of chemical reactions, including polymerisation reactions, between adjacent molecules in crystals, and such processes are bound to be modified if the state of positional and orientational order of molecules can be varied and then frozen into a particular pattern. Perhaps compounds can now be found in which internal reaction mechanisms will change at glass transition temperatures. If this proves to be so, we shall need a new definition to denote the process: vitrochemistry, perhaps?

Behaviour in Europe

from a Correspondent

THE first European Neurosciences meeting will be held in Munich next September (for details see the advertisement on page vii). This meeting represents the culmination of seven years' effort to promote collaboration between workers in different European countries and in different disciplines within the field of brain and behaviour research. The meeting is organised by the European Training Programme in Brain and Behaviour Research (ETP), a body whose history is of some interest.

In 1968, the Organisation for Economic Cooperation and Development inaugurated studies of three scientific fields: the criteria for selecting fields were that they should be multi-disciplinary, have a fast growth rate, have insufficient recognition, and

Climbing the technology tree

from Andrew Holmes-Siedle

THE Institute of Electronic and Electrical Engineers, one of the largest professional societies in the world, has turned some of its effort to helping those scientists and engineers faced with redundancy to plot their careers in such a way that they avoid the trap of 'irrelevance'. Its Technology Forecasting and Assessment Committee, whose original purpose had been to advise industry and government on planning, has now turned all its attention to an advanced form of career guidance. The committee has picked areas of application for which technology is being developed intensively and drawn

for each a 'technology tree' which relates these areas logically and lists the technologies involved in each.

To give an example, the Nuclear and Plasma Sciences Tree (Published in the IEEE Nuclear and Plasma Sciences Society Newsletter, October 1974) carries a limb entitled 'Radiation Effects' which contains eleven branches with 43 twigs, the subjects ranging from charge loss in capacitors to food preservation. To put it another way, the IEEE has identified some technological bandwagons and given us a few clues as to how we might climb on; the rest is up to us.

be likely to produce results both of scientific and practical importance within a decade. One of the fields chosen was Brain and Behaviour and in 1972 OECD published a report with this title prepared by two of its consultants, Dr Otto Wolhuis and Professor Stuart Sutherland. The report summarised the scientific status of the field and analysed a number of defects in its organisation in Europe including Britain. Among the defects mentioned were the lack of mobility of scientists, resulting in a slow flow of ideas and parochial journals; lack of communication between European countries; small laboratories with insufficient interaction between, for example, neurophysiologists and psychologists; and authoritarian departmental structures.

The OECD investigation led in 1970 to the setting up of ETP with a generous grant from the Max-Planck-Gesellschaft. It now receives contributions from Austria, Britain, France, Germany, Italy, Netherlands, and Switzerland and seven additional countries have representatives on the steering committee and participate in the programme. So far funds have been used for the following purposes:

- The training of young scientists in countries other than their own and usually in disciplines other than their primary subject.
- Four highly successful winter schools for young scientists from all European countries—again with a multi-disciplinary emphasis.
- 'Twinning' grants awarded to about 100 laboratories, enabling the members of each to travel to a sister laboratory abroad which is engaged on complementary work.
- Grants enabling young scientists to attend conferences abroad organised by bodies other than ETP.

In the five years of its existence ETP, operating on an insecure budget of about £50,000 a year, has done much to promote cooperation between workers in brain and behaviour in different European countries and in the different component disciplines. It is hoped that when the European Science Foundation starts functioning ETP will become affiliated, and that a more permanent flow of funds will be secured through international treaties. Unlike EMBO, ETP has set its heart against the founding of a large international laboratory since it is thought that the centralisation and bureaucracy involved are not in the best interests of the subject.

High-pass phonon filter

from P. V. E. McClintock

SUPERFLUID ⁴He can be made to act as a tunable high-pass phonon filter, according to some recent experimental work at Nottingham University by Wyatt, Lockerbie and Sherlock (*Phys. Rev. Lett.*, **33**, 1425; 1974).

Their experiment was performed below 0.1 K, where liquid ⁴He is an almost pure superfluid, that is, a fluid which can indulge in frictionless flow, or through which objects can move without any dissipation of their kinetic energy. The superfluid itself carries no entropy, but acts as the inert background or aether supporting a gas of phonons, which are the quanta of vibrational energy. These behave much like a collection of independent particles moving at the velocity of sound, and they carry all the thermal energy of the liquid.

The Nottingham experiment consisted, essentially, of injecting relatively

small numbers of very high energy phonons into the superfluid at temperatures below 0.1 K, investigating how they propagated through the liquid and, in particular, deducing whether they were able to decay. (Strictly, of course, the term 'temperature' is not applicable once the high energy phonons have been injected, because there then exists a non-equilibrium situation.)

Considerable interest attaches to whether or not a phonon of given initial energy is able to decay into two phonons of lower energy, since this yields direct information about the shape of the dispersion curve, that is, the relationship between a phonon's energy ϵ and its momentum p . It was long believed that for small ϵ the dispersion curve was linear, of the form $\epsilon = cp$ where c is the phonon velocity in the long wavelength limit, but that at higher ϵ the curve deviated below cp (negative dispersion), representing smaller phonon velocities. More recently, mounting evidence has apparently indicated that, although the dispersion curve is indeed linear at very small ϵ , there is an intermediate regime in which it deviates upwards (positive dispersion) before entering the negative dispersion region.

The precise shape of the dispersion curve is of considerable importance because of its profound influence on 3-phonon processes, that is, the decay of one phonon into two of lower energy or, conversely, the combination of two low energy phonons to create one high energy phonon. Such processes are of crucial importance in determining many of the properties of the liquid, such as the ultrasonic attenuation or the normal fluid viscosity, at temperatures near 1 K. It turns out that for 3-phonon processes it is only possible to satisfy simultaneously the conservation of energy and momentum in the presence of positive dispersion: the interactions cannot therefore occur at all where the dispersion is negative. Unfortunately, it is difficult to deduce the shape of the dispersion curve unambiguously from, for example, normal fluid viscosity measurements near 1 K. This is partly because phonons of a very wide range of energies are simultaneously present, but also because of complications arising from the existence at these relatively high temperatures of large numbers of rotons, which are another type of thermal excitation.

Hence the value of performing experiments at very much lower temperatures where the thermally excited 'background' phonons are so few that they can be neglected. The Nottingham group used a superconducting fluoroscer to inject into the liquid pulses of phonons which all had energies larger than 2Δ (Δ being the energy gap of the superconductor), and detected

the phonons again after they had travelled several mm through the liquid with a superconducting tunnel junction, a device which is sensitive only to phonons of energy greater than 2Δ . Thus, the decay of an injected phonon would have yielded two phonons each of whose individual energies would have been too small to be seen by the detector, so that the observed attenuation of the phonon pulse could be regarded as a direct measure of the extent to which 3-phonon decays had occurred during the transit.

The main experimental finding was that the number of phonons reaching the detector depended strongly on pressure for pressures from 0 to 13.5 bar, but was almost pressure independent from 13.5 bar right up to the solidification pressure of 25 bar. The effect could not be due to changes in the number of high energy phonons generated in the fluorescer, since the performance of these devices is independent of pressure. There appeared to be two possible explanations: (1) some sort of dissipative effect occurring in the bulk of the liquid, attenuating all phonons; or (2) because of positive dispersion, the injected phonons might have been able to decay by 3-phonon processes, resulting in phonons of insufficient energy to stimulate the detector.

The first hypothesis was rapidly disposed of by altering the separation of the source and detector. The pressure dependence of the detector signal was quite independent of the separation, thus ruling out possible bulk attenuation effects.

The authors therefore conclude that the second hypothesis is the correct explanation. They suggest that there is a critical phonon energy ϵ_c below which a phonon decays rapidly, but above which a phonon will be stable and therefore able to travel long distances through the liquid. If ϵ_c decreases with increasing pressure, and if at 13.5 bar ϵ_c is equal to the minimum injected phonon energy of 2Δ , then the experimental data can all be understood. At higher pressures ϵ_c would become smaller than the lowest energy phonon from the fluorescer and the injected phonons would therefore all reach the detector without decaying, thus accounting for the observed pressure independence of the signal above 13.5 bar.

The scenario proposed by the authors is therefore as follows. When a pulse of phonons with an initially wide range of energies is injected into the superfluid, those phonons with energies less than ϵ_c decay almost immediately owing to the existence of positive dispersion, whereas those with higher energies, in the negative dispersion regime, propagate freely. Thus the liquid acts as a

high pass filter, passing only phonons of energy above ϵ_c . Because ϵ_c can be changed by altering the pressure, the filter is tunable and clearly has possible applications in the growing field of phonon spectroscopy.

This rather novel picture of liquid helium is certainly consistent with existing experimental information, but it is to be hoped that the authors will now carry out further checks on its veracity, in particular by repeating the experiments using detectors sensitive to a number of different phonon energies.

Structure and dynamics of spiral galaxies

from Vincent Icke and James Pringle

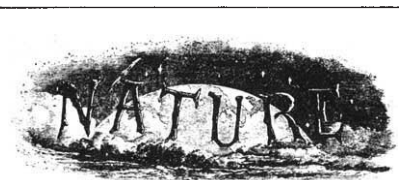
On Thursday, January 9, an informal discussion day at the Institute of Astronomy, Cambridge, brought together some 50 astronomers from various parts of the UK, to exchange news and views on the structure and dynamics of spiral galaxies.

BARRY MADORE (IOA, Cambridge) reviewed the situation nearest home, showing how the spiral structure of our Galaxy in the neighbourhood of the Sun can be determined by 'spiral tracers'. These are young, massive bright stars which, since star formation takes place predominantly in spiral arms, serve as 'standard candles' to delineate spiral structure out to distances of about 15,000 light years. Observing at Cerro Tololo, Madore has concentrated on the Northern Hemisphere which thus far has received only thin coverage. His results on the distances of classical Cepheids has extended the known nearby spiral pattern, but more observations are still required.

Passing to external galaxies, Greg Davidson (Jodrell Bank) and Darrell Emerson (Mullard Radio Astronomy Observatory, Cambridge) presented observations of the rotation speed and the neutral hydrogen distribution of the Andromeda Nebula M31. The dependence of the rotation speed on radial distance indicates the mass distribution in a galaxy. Davidson found that in the outer regions of M31, the rotation curve is flat, indicating a very large amount of mass in the 'halo' of this galaxy. The results, however, are not yet clear cut because the low resolution of the Jodrell Bank Mk I telescope tends to smear out and flatten the rotation curve. Emerson's observations of the disk showed ridges of condensed gas around the centre of M31,

forming rings or spiral arms (this galaxy is seen too edge on to enable one to say which of these two alternatives is the better). The velocity field, mapped by observing the Doppler shifts of the 21 cm hydrogen line, shows distinct non-circular motions, perhaps indicative of the motions which theorists predict for the gas condensations as spiralling density waves propagating through the disk. But here too the galactic inclination makes it impossible to distinguish between spiral and ring-like structure.

An alternative to density waves as the cause of spiral structure is the possibility that spiral arms are formed by galactic 'tides', generated by a previous close encounter of two galaxies. Observational evidence for such encounters was given by Anthony Winter and Geoffrey Cottrell (MRAO, Cambridge) who have respectively studied the pairs NGC9631-9656 and M81-NGC3077. An extensive neutral hydrogen complex is found to surround the spiral galaxies NGC9631-9656. From the velocity structure of the complex it is apparent that the gas actually links the two galaxies; the velocities in the gas match those of the galaxies and show a systematic trend from one to the other, thus linking them in 'velocity space'. Although the observations clearly show that the encounter profoundly disturbs each member of the pair, the influence on spiral structure is unknown because both galaxies are seen edge on. The pair M81-NGC3077 is more promising in this respect: M81 is one of the most beautiful two-armed spiral galaxies known. In this case, however, the evidence for tidal interaction is less strong. NGC3077 possesses a comma-shaped hydrogen extension, pointing towards M81, and this 'tail' may be a remnant of a hyperbolic encounter about 10^9 yr ago.



A hundred years ago

IN reference to the proposed Channel Tunnel between France and England, we may refer our readers to *NATURE*, vol. i., pp. 160, 303, 631, and vol. x., p. 181, where the scientific bearings of the subject are pretty fully discussed. While on this matter we may state, on the authority of *La Nature*, that there has been in existence for some time in Spain an Inter-continental Railway Company, whose object is to connect Europe and Africa by a tunnel underneath the Straits of Gibraltar, the maximum depth of which is 819 metres.

from *Nature*, 11, 273; February 4, 1875

To understand the large-scale motions in galaxies it is important to know how they interact with their surroundings. An aspect of this was studied by Roland Hunt (University of Oxford) who presented theoretical calculations of the effects which the infall of intergalactic gas would have on our Galaxy. The main consequences are a slow inward streaming of gas in the galactic plane and a peculiar relationship between the chemical composition of stars and their ages. The observational data are consistent with the no infall of gas at all, but an accretion rate of three solar masses per year is not excluded.

James Binney (University of Oxford) discussed a problem which has confronted theorists almost since the discovery of external galaxies, namely the origin of their considerable angular momentum content. In a universe which is initially homogeneous and isotropic (as ours appears to be) vorticity must somehow be generated. Binney has considered the creation of vorticity by the shock fronts which probably accompany the early stages of contraction of clusters of galaxies. It seems possible to account for the rotation of spiral galaxies, but the model may be rather sensitive to the shape of the shock fronts.

For simplicity, most theorists assume that the motions in a spiral galaxy can be satisfactorily simplified to motion in an infinitesimally thin disk. Douglas Heggie (IOA, Cambridge) presented some surprising results which indicate that one should be wary of such implications. He finds that, contrary to earlier expectations, the gravitational potential of a spiral wave can excite stars into resonant orbits perpendicular to the galactic plane. This drives stars far away from the plane, giving the disk a definite thickness. Resonance of the stellar orbits with the spiral pattern can then interfere with the propagation of spiral waves.

How a spiral wave might persist with a constant amplitude over the $\sim 10^{10}$ years' lifetime of a galaxy was discussed by Vincent Icke (IOA, Cambridge). He presented a wave equation in which energy losses and dispersive effects are counteracted by nonlinear terms, so that some wave solutions maintain the same amplitude indefinitely. Analogous equations in hydrodynamics and plasma physics exist which also exhibit these solitary waves or 'solutions'. Finding two-dimensional solutions, however, seems difficult.

Bernard Schutz (University College, Cardiff) presented calculations of the normal modes of instability of a galactic disk. In this type of analysis, the solution of Poisson's equation for the gravitational potential and of Vlasov's equation for the velocity distribution of the stars present formidable

obstacles. These were side-stepped by exploiting the thinness of the disk to approximate the gravitational potential by a polynomial series, and by treating the disk as a fluid. It is found that the fastest growing mode is a very open spiral, trailing with respect to the galactic rotation.

A computational approach to the origin of spiral structure was made by David Brownrigg (University of Reading), who used a cell-averaging technique to calculate the three-dimensional motion of 25,000 particles with mutual gravitational attraction. These particles represent Population I disk stars moving in a spherical potential corresponding to the average gravitational field of the Population II halo stars. The model seems to be a fair representation of a self-gravitating collisionless point-particle system. The model galaxy exhibits some impressive density-wave type spiral structure in its outermost parts.

Present thinking on the problem of galactic spiral structure was clearly reflected in the emphasis which the participants in the discussion put upon the observation of noncircular motion in single galaxies and of interacting galaxies, and in the preoccupation of most theorists with instabilities and wave propagation in differentially rotating disks. It seemed to be generally felt that progress in the understanding of spiral galaxies will be conditional upon the progress in these fields.

Identifying ancient forest

from Peter D. Moore

THE climatic climax vegetation of England and Wales is generally considered to be deciduous forest, yet, as a result of human interference since prehistoric times, little, if any, woodland with an uninterrupted history now remains. It is natural, therefore, that conservationists should be concerned with devising methods by which fragments of ancient forest can be identified in order to protect them from further damage. The most useful criteria so far employed as indices of antiquity are the general species density of the habitat and the presence of species which are sensitive to disturbance.

Species richness is a concept which was touched upon recently in these columns when its relationship to time was considered (*Nature*, **252**, 14; 1974). Southwood (*J. anim. Ecol.*, **30**, 1; 1960) demonstrated a positive correlation between subfossil abundance of British tree species and the number of associated insect species. He also demonstrated from Hawaiian data (*Proc.*

Hawaiian ent. Soc., **17**, 299; 1960) that a general relationship exists in which the more abundant trees have higher numbers of associated insects. It is reasonable to expect, therefore, that assemblages of tree species which have occupied extensive and uninterrupted areas over considerable periods should support a rich insect fauna. It would seem reasonable also to extend this argument to associated plants and to use, for example, the richness of epiphytic bryophyte or lichen flora or of understorey herbs as an indication of old forest.

Rose and James (*Lichenologist*, **6**, 1; 1974) have recently studied the lichen flora of the New Forest in southern England. This ancient Royal Hunting Forest contains wooded areas which have remained forested at least since mediaeval times and probably for even longer. The epiphytic lichen flora of the forest was found to be exceptionally rich, 259 taxa being recorded during the intensive six-year survey. Species density in some areas is as high as 160 km^{-2} which is a figure exceeded only in some extremely oceanic woodlands in the western extremities of Britain (Rose, in *The British Oak; Its History and Natural History*, edit. by M. G. Morris, and F. H. Perring, 258; Classey, Farringdon, 1974) where climatic factors are particularly suitable for epiphyte growth. It appears, therefore, that epiphyte species density can provide some measure of the historical continuity and lack of fragmentation of a forest.

The richness of the epiphyte flora of the New Forest cannot be explained simply in terms of the tree species available as hosts. Many epiphytes are selective with respect to host species, being particularly sensitive to the physical and chemical nature of the host's bark. Rose has determined the number of epiphytic lichens associated with various tree species and they fall in the order: oak (303), ash (230), beech (194), elm (171) and sycamore (170). The New Forest contains mainly oak and beech, but the other epiphyte-rich tree species are rare or absent. Since oak/beech forest is common in southern Britain, the richness of the New Forest is best explained in terms of its historical continuity and also the low levels of atmospheric pollution experienced.

It is interesting to note in passing that the richness of Rose's epiphytic lichens is not related to the age or to the area of trees, or both, in the way that Southwood has demonstrated for insects. Some of our more ancient and widespread trees, for example, have a far poorer epiphyte flora than those mentioned above, such as birch (93), hazel (124) and alder (72). Southwood has suggested that an abundant tree is

more frequently encountered by an insect and that this assists the development of a feeding relationship. This process does not seem to have been generally operative in the case of the host/epiphyte situation during the post-glacial period in Britain.

Species density alone, therefore, may indicate antiquity in a forest, but it may also be misleading under certain conditions; for example, the increased microhabitat diversity which accompanies limited disturbance may, at least temporarily, inflate species density since it allows the invasion of species characteristic of unstable habitats. Also, local air pollution may deplete the epiphyte flora of even an ancient forest. A more hopeful approach to the assessment of past forest continuity is the use of species which are sensitive to disturbance. Rose has attempted this by constructing an "Index of Ecological Continuity" for woodlands based upon the occurrence of twenty selected lichen species which he regards as indicative of long periods of undisturbed woodland conditions. On this scale the New Forest has a rating of 100; very few other British woodlands have values of more than 70. Again, however, some woods with lengthy historical continuity could be underrated because of recent pollution, particularly in eastern Britain.

Peterken has recently proposed (*Biol. Cons.*, 6, 239; 1974) a similar system which he has used for the assessment of woodlands in Lincolnshire for conservation purposes. He selects flowering plants and ferns confined or almost confined to proved ancient ('primary') woodlands within an intensively surveyed study area and uses these as a yardstick for assessing woodlands over a wide region. His data demonstrate a positive correlation between the number of 'primary' woodland species recorded at a site and the area of the site. This suggests that richness in sensitive woodland understorey species is a function of undisturbed conditions in both temporal and spatial dimensions. As with the epiphytes, the oceanic conditions of western Britain are particularly favourable for the growth of woodland plants, hence some of the species indicative of antiquity in eastern Britain are more widespread in the west. Complications from air pollution, however, are likely to be less important than when using epiphytes as indicators, since understorey herbs are less sensitive.

Many of the plant species used by Rose and by Peterken as indicators of undisturbed situations grow slowly under conditions of low humidity and are poor in their dispersal capacity; thus spatial fragmentation of woodland presents them with problems of spread and survival. It is the presence of these

indicators, rather than the total number of species per unit area, which is likely to provide the clues for the detection and subsequent conservation of our dwindling fragments of ancient woodland.

Properties of plasmas

from A. G. Sitenko

The Second International Conference on Plasma Theory was held in Kiev from October 28 to November 1. The Conference was organized by the Institute for Theoretical Physics of the Academy of Sciences of the Ukrainian SSR and the Lebedev Physical Institute of the USSR Academy of Sciences and was sponsored by the International Union of Pure and Applied Physics.

CONTEMPORARY plasma theory is based largely on statistical methods—but there are different techniques for describing the properties of the statistical system. A. G. Sitenko proposed a detailed description of statistical and electrodynamical plasma properties, based on the consideration of fluctuations. Of fundamental importance in this description are the spectral correlation functions, which have simple analytical properties. In linear electrodynamics the fluctuation-dissipation theorem establishes a relation between fluctuations of different quantities and electrodynamic properties of the medium. That is why, once we know the fluctuation spectrum in plasmas, we can determine the electric plasma susceptibility by inversion of the fluctuation-dissipation theorem. The generalisation of this theorem for the non-linear case allows the description of non-linear electrodynamic plasma properties. Allowing for the non-linear wave interaction explains the saturation of the fluctuation level in non-equilibrium plasma under critical conditions. This level, corresponding to the stationary turbulent plasma state, can exceed the thermal level significantly. The kinetic equation for waves is derived, taking into account the interaction of waves with fluctuating fields in plasmas. To neglect this interaction (a procedure adopted in many papers) is not valid in nonequilibrium plasmas. In the stationary case the solution of the kinetic equation determines the fluctuation spectrum for the turbulent plasma state. Such an approach is very promising when considering the scattering and transformation of waves in plasmas.

V. E. Zakharov applied the inverse scattering problem method to non-

linear problems of plasma physics. He showed that a set of non-linear equations, which describes resonant interaction of three one-dimensional wave packets, may be solved exactly by the inverse problem method. The conservation laws are found and complete integrability of the equations is proved. In the case of decay resonant interaction between wavepackets the physical picture depends on the relative magnitudes of pumping rate and secondary waves' velocities: if the pumping rate is intermediate between the velocities of secondary waves, then the long packet of pumping decays practically completely when it collides even with arbitrarily small secondary wavepackets; but if the pumping rate is extreme, then the decay of the pumping wave is possible only when colliding with intense enough secondary wavepackets. The solutions for explosive instability are found, which describe the rise of local singularities in the limited wavepackets.

The question of turbulent plasma heating by beams of electrons and light was discussed by L. I. Rudakov on the basis of the soliton model of turbulence. The solitons may be formed as a result of a one-dimensional turbulent state modulation instability and are in fact sets of waves, trapped by the diminished plasma density domains, which, in turn, appear under the high-frequency field pressure. The formation of solitons is energetically advantageous, as it causes a decrease of the Langmuir quanta frequency, and the released energy promotes the formation of diminished density domains. As a result of collisions the solitons can decay or join. If the distances between the solitons exceed their sizes, then binary collisions play the main part. The electron changes its velocity when passing through the soliton. As a result of repeated collisions the electrons can increase their energy, thus changing the distribution function. This picture for the one-dimensional case is confirmed by the numerical integration of the non-linear equations of Langmuir turbulence. The joining of solitons can lead to the collapse phenomenon, that is, to dynamical accumulation of energy in certain regions of space. The existence of such a process, when special initial assumptions are fulfilled, is confirmed by numerical calculation. The interaction between particles and the collapsing formation causes the heating of the particles in the medium.

In conclusion, the conference gave participants a good chance to acquaint each other with investigations in plasma theory. The next International Conference on Plasma Theory will be organised by A. Salam in 1976 at Trieste.

review articles

Unclocklike behaviour of biological clocks

Arthur T. Winfree*

The mechanisms which could underlie circadian rhythms fall naturally into groups with qualitatively different responses to disruption. Experiments designed to distinguish between these mechanisms seem to exclude all of them. It may be that multicellular organisms keep time, not by one 'clock' but by averaging many independent circadian oscillators.

"I hear the sound of time. And yet I *slew* it, and wiped my bloody sword upon its beard."¹

AN impressive diversity of organisms seems to have internalised the 24-h periodicity of this rotating planet. Exceptions include the prokaryotes, the bryophytes, most embryos and species evolved in soil, deep caves and the abyss²⁻⁶. But in most other creatures, body temperature, enzyme activity, leaf movement, neural firing, mitotic index or other convenient observables typically persist in regular up and down periods of about 24-h, even in ostensibly constant conditions²⁻⁶. These are called circadian rhythms to distinguish them from biological activities which persist in 24-h periodicity only so long as they are exposed to environmental cues at the same intervals. Brown and coworkers argue that even these circadian rhythms would stop or grossly change their period should the planet stop rotating, because they depend on the organisms sensing rhythmic fluctuations in subtle geophysical variables²⁻⁶. Although some quantities, such as the electrical charge of the atmosphere, fluctuate diurnally in synchrony around the whole planet⁷, many candidates for the role of covert driving rhythm are excluded by the persistence of circadian rhythms on a rotating platform at the south pole⁸. Though it is impossible in principle to exclude all hypothetical drivers experimentally, most workers since the 1930s^{8,9} infer from the following generalisations that one ought instead to look for a mechanism of spontaneous oscillation within the organism.

The circadian period, though often precise to within an hour per month, seldom exactly matches the dominant periods of geophysical variables. The circadian period depends on temperature, typically drifts a few percent within one day and is chemically adjustable. Mutants of altered period exist. The phase of a circadian rhythm is labile: with few exceptions (possibly including humans) it is permanently reset by a pulse of light or brief elevation of temperature within the physiological range or by temporarily withholding oxygen²⁻⁶.

Moreover, most workers seem to think in terms of an ultimately cellular mechanism, because organ and tissue-level interactions do not seem to be crucially involved.

Protozoa and unicellular algae exhibit typical circadian rhythms (for example of bioluminescence, motility, mitosis and surface antigenicity). The leaves of plants, bits of cut leaf or of cultured leaf callus independently support circadian rhythms of movement or respiration. Isolated mammalian organs and even disaggregated cells persist in

circadian variations of electrical activity, enzyme activity, respiration and so on²⁻⁶.

Attempts to pinpoint the cellular mechanism of such long-period rhythmicity have led to models emphasising cellular membranes¹⁰, cytoplasmic enzyme reactions¹¹⁻¹³, or sequential transcription of the nuclear message¹⁴⁻¹⁶. The diversity of action spectra for rephasing by light²⁻⁶ suggests a corresponding diversity of molecular mechanisms, at least at the photoreceptor end of 'the clock'.

Without wishing to deny the fascination of particular cases, I feel that a biophysical search for the unique mechanism of some universal clock is likely to be no more rewarding than earlier quixotic searches for the mechanism of 'homeostasis' or of 'excitability'. In such complex dynamical systems as living cells, stable steady-state operation can only be a result of the most intense selection¹⁷. Instabilities typically lead to spontaneous oscillation (the alternatives being some kind of semiperiodicity or unpredictable aperiodic fluctuations¹⁸). Thus the role of natural selection in adapting cells to a periodic milieu may be not to contrive and refine a timer so much as to weed out irregular fluctuations and oscillations of maladaptive period and to synchronise the rest. Such speculation discourages belief in a unique evolutionary ancestor of contemporary 'clocks'. It offers an interpretation of the curious facts that many circadian rhythms lack discernible selective value^{6,19,20} and that their evolution seldom if ever involved selective challenges during prolonged isolation from the environmental day/night cycle ... yet they cycle autonomously with precise period. It may be that the generic features of circadian rhythms stem more from convergent evolution and the common properties of complex dynamical systems than from a ubiquitous mechanism. For example, many of the peculiarities of the phase-resetting behaviour of circadian rhythms are also seen in a wide variety of electronic²¹, mechanical, and mathematical models²⁻⁶, and can be rationalised by simple topological principles without recourse either to such metaphorical models or to arguments from presumed selective advantage²².

What then can be learned by studying the phase shifts of biological rhythms? Such study leads to some 'unclocklike' aspects of whole-organism rhythmicity which were neglected so long as the "clock" metaphor, early introduced²³⁻²⁵ to emphasise the presumed significance of circadian rhythms for timing, biased investigators toward preoccupation with phase on a presumed rigid cycle. The encounter with unexpected and 'unclocklike' behaviour results from investigations into that timing role and the adaptive reasons for it.

Given the inevitable small discrepancy between the organism's circadian period and that of the environmental

*Address: Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907.

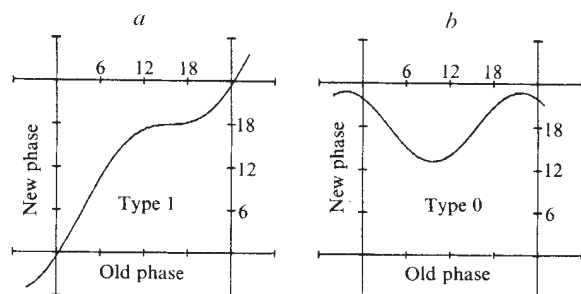


Fig. 1 Old phase and new phase axes each extend through 1.5 cycles of 24 h. *a*, A type 1 resetting curve (typical of 'simple clock' mechanisms and weakly perturbed limit cycle mechanisms) rises through 1 cycle of new phase per cycle of old phase. *b*, A type 0 resetting curve (typical of strongly perturbed limit cycle mechanisms) shows no net drift along the new phase axis.

rhythms of food availability, predation, warmth, dryness and so on, an occasional phase correction seems necessary to maintain any preferred phase relation. The ultimate pacemaker and most reliable indicator of environmental phase is the rising and setting of the sun, that is, the light/dark cycle. With few exceptions, circadian rhythms are temporarily deranged by visible light: when normal rhythmicity recovers, it is found to be phase-shifted relative to an unexposed control. In *Drosophila*, for example, light as dim as starlight, if prolonged, damps out the rhythm²⁶; and a brief exposure at somewhat greater intensity changes the phase by as much as half a cycle before even 1% of the pigment molecules can have absorbed a photon²⁷. (The clock pigment of *Drosophila* is not in its eyes and is not rhodopsin (refs 28 and 29 and A. T. W. unpublished).) By altering the normal light cycle in agricultural fields with dim lights³⁰ or even a single nightly photoflash³¹, the seasonal adaptations of insect pests can be lethally disrupted, presumably through circadian rhythms involved in seasonal photoperiodism^{32,33}.

To ensure stable entrainment, the phase adjustment inflicted must vary according to the original phase at exposure. Measurements of this dependence have been carried out in many laboratories investigating the cell cycle³⁴, neurophysiological rhythms³⁵⁻³⁷ and circadian rhythms²⁻⁶. Particularly in the latter cases, certain peculiar regularities have emerged which seem hard to account for in terms of natural selection, but are typical of a broad class of dynamical systems (and atypical of others). Before introducing these data it will be useful to distinguish three traditions of thinking about the mechanism of biological rhythms.

Clocks and oscillators

In the first view, the mechanism underlying overt rhythmicity is called a 'clock' or, as in the cell cycle literature, a 'simple clock'^{38,39}. Its possible states can be arranged in a recurring sequence in which each state induces the next in a repeating cycle. The "state space" is a circle and there is no state which is not represented in that space. The clocks of home and industry are of this kind; the only variable quantity in the clock is the angular position of its meshed gears, and to get to a new position, it must pass forward or backward through all intermediate positions. Virus replication constitutes such a clock, the polymerase advancing round and round a circular genome at a rate limited by substrate availability; a similar model originally proposed for the cell cycle⁴⁰ is the basis of one conjecture about the circadian mechanism in eukaryotic cells^{14-16,41}.

Much of the literature implies that 'biological clocks' are clocks in this sense, that is, that the mechanism adheres to

a one-parameter sequence of states or 'subjective circadian times'. For example the widely adopted definitions of "phase" and "phase shift"⁴² and many analyses of the process of rephasing⁴³⁻⁴⁵ implicitly or explicitly assume that the clock is always at some phase in its cycle, with phase changing at a rate determined by light intensity and current phase. Integrating this rate function as the phase changes during an exposure, one can calculate the new phase reached as a function of old phase for a given light intensity and duration⁴³⁻⁴⁵. 'Simple clock' mechanisms are distinguished by the smoothness of this rephasing curve (Fig. 1*a*), its limitation to positive slopes and the fact that its mean slope is necessarily exactly 1 (as it is, for example, in response to a vanishingly brief stimulus, following which new phase = old phase)³⁹. We call this, by the mean slope of the rephasing curve, 'type 1' rephasing: the curve rises through one cycle of new phase in each cycle of old phase it traverses to the right.

In contrast, Kalmus^{9,46} and Mori⁴⁷, following Bonhoeffer's treatment of electrical rhythms in cells^{48,49}, were the first to emphasise limit-cycle oscillator models for circadian rhythms. In this view two or more variables (for example chemical concentrations) smoothly affect each other's rates of change in such a way that departures from equilibrium are not opposed. Either spontaneously, or once disturbed beyond a certain threshold distance from equilibrium, such a system quickly approaches a 'limit cycle' in which both quantities fluctuate regularly, at the same period but out of phase. Disturbed from this cycle, the system quickly returns to it; amplitude and waveform are subject to vigorous regulation. Regardless of their potential complexity (for example the number of variables involved), limit cycle mechanisms differ from 'simple clock' mechanisms in the possession of easily accessible states, (including an equilibrium point) from which normal rhythmicity

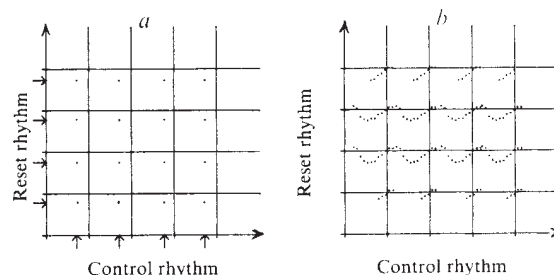


Fig. 2 Cycles 4-8 of control rhythm event time are plotted against reset rhythm event time, both measured from stimulus time. Since event time (arrows in *a*) is the complement of phase at stimulus time, these axes are equivalent to backward old phase and new phase axes, respectively. *a*, Plot of a single experiment against its control; *b*, (arrows omitted) twelve experiments plotted, each with stimulus falling at a different phase in the control rhythm. Data represent centroids of *Drosophila* eclosion peaks, showing type 0 reset by 12,000 erg cm⁻² of blue light.

never develops, or does so at unpredictable phase after an unpredictable lag period^{19,50,51}. These phenomena are implicit in several mathematical metaphors employed by theorists of circadian rhythmicity^{52,53}. The rephasing curves of limit cycle mechanisms are not limited to positive slopes, and can have a mean slope of 1 or 0 (or any other integer if more than 2 variables are involved)¹⁹. In 'type 0' rephasing, what goes up must come down before the curve passes one cycle of old phase to the right (Fig. 1*b*).

And what about the ubiquitous relaxation oscillator model? According to this view, popular among biologists concerned with the cell cycle³⁴ and nerve activity⁵⁴ as well as circadian rhythmicity^{2-6,55}, some photolabile metabolite accumulates until a threshold concentration is reached, when it is all destroyed or a gradual destruction begins

which will be terminated at a lower threshold. Exposure to light or a temperature shock prematurely destroys the accumulated substance. Though such a mechanism may be viewed as a limiting case of a simple clock or limit cycle, it is conveniently treated separately. Its rephasing curves all have an abrupt jump whose magnitude increases with stimulus duration. Consequently 'average slope' is ambiguous.

Simple clock excluded experimentally

No circadian mechanism has been observed directly. But it is possible to measure the rephasing curves by monitoring any conveniently observable rhythm, on the assumption that a constant phase difference between an undisturbed control and the disturbed rhythm reflects the same phase difference between the circadian oscillators driving each rhythm (usually it is a few cycles before the disturbed rhythm recovers its normal period and the phase difference becomes constant). Taking the time of the rephasing stimulus as zero and plotting recurrent event times in the reset rhythm against control event times recurring at the same period, a square lattice of data points can be obtained (Fig. 2a). Superimposing many experiments, in which the stimulus struck at all phases of the cycle, one obtains a square lattice of rephasing curves (Fig. 2b). The plot is periodic along both time axes. Each unit cell of this 'time crystal' contains as many data points as there were experiments, typically arranged along a smooth rephasing curve that scans some integer number (including 0 as a possibility as in Fig. 2b) of cycles vertically as it passes one cycle horizontally. This smoothness is the first fact. It eliminates relaxation-oscillator models, for which an abrupt jump in the rephasing curve is expected at every stimulus duration. (The "breakpoint" or "point of no return"^{3,5} is often depicted in "response curves"—phase shift against original phase—by a data-less vertical dashed line spanning one cycle. This indicates the phase at which the predominant direction of short-term transients in the upset rhythm changes from delays to advances. This typically occurs where the eventual steady phase shift is close to a half cycle. There is no such jump in the rephasing curves.)

The second crucial fact is that every species shows type 1 rephasing in response to sufficiently faint stimuli. This is because a faintly reset rhythm is almost identical to its control, so plotting one against the other gives data points near the rising diagonal in the time crystal. Thirdly, curves with broad regions of negative slope and mean slope 0 are not uncommon and might even occur in all species, given a sufficiently potent stimulus. This eliminates 'simple clock' cycles in all species showing type 0 rephasing.

The fourth crucial fact emerges when the 'time crystal' is extended into three dimensions along a stimulus duration axis. The rephasing curves extend into wavy surfaces, but not separate surfaces as expected of a simple clock mechanism. One finds instead a single corkscrew-shaped surface winding around a vertical symmetry axis (Fig. 3). At this isolated singular phase and duration of stimulus, the repeated surfaces connect together by tilting vertically, so that new phase is ambiguous. Following this stimulus, circadian rhythmicity is abolished or only recovers with random phase after an unpredictable lag period (Fig. 4). The rephasing curves for stimuli exceeding this critical duration have mean slope 0; those for briefer stimuli have mean slope 1. This is the picture as it emerges in the only two species for which measurements have been carried out in sufficient detail, that is *Drosophila*^{22,27,39} and the succulent plant *Kalanchoe*^{36,37}. Fragmentary evidence from a diversity of other circadian systems, perturbed by diverse rephasing stimuli, seems to fit the same pattern^{38,39}. This is the pattern expected for limit-cycle mechanisms⁶⁰.

The existence of a singularity reveals states not on the 'clock's' cycle, and establishes the importance of some second and uncontrolled experimental variable, complementary to the 'phase' or 'subjective circadian time' which has apparently sufficed up to now for description of experiments and inference from their results. Perhaps some of

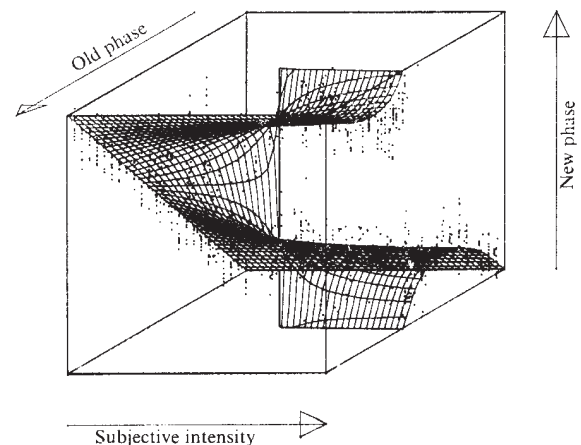


Fig. 3 The curved surface depicts new phase (from 0 to 24 vertically) against old phase (from 0 at the rear to 24 at the front) and subjective stimulus intensity (from zero to the right) for the circadian eclosion rhythm of *Drosophila*, as calculated from the simplest oscillator model²⁷. Phase is recorded as h from eclosion peaks (extrapolated back in steps of 24 h) to stimulus time. The 1,327 data points represent eclosion peak centroids of clearly rhythmic populations ($R < 50$)⁶⁰, in experiments covering four cycles of old and new phase, all reduced to this one unit cell. Subjective stimulus intensity is exposure energy divided by an exponential dark-adaptation factor. Note that exposure during an eclosion peak (phase 0, the front and back edges) does not affect subsequent eclosion timing; and exposure midway between peaks at a critical intensity results in ambiguous phase (the vertical pole around which the surface and data climb). The data and surface in this unit cell would fit smoothly on to replicas above, below, in front, and behind.

those inferences could now be re-examined. A similar dilemma seems to be arising through Kauffman and Wille's application of similar experiments^{30,61} to the cell cycle: the familiar 'simple clock'³⁸ and 'mitogen relaxation oscillator' interpretations³⁴ of cellular control over mitosis provide no interpretation for a singularity or for type 0 rephasing.

Limit cycle excluded experimentally

In the singularity we are confronted with a first 'unlock-like' aspect of circadian chronometry. A second emerges from the attempt to pursue the idea of a quickly recovering limit-cycle mechanism through the entrainment of circadian rhythms by stimuli repeated at 24-h intervals.

Perkel *et al.*³⁵ carried out an elegant theoretical and empirical analysis of entrainment for pacemaker neurones exposed to regular synaptic input at millisecond intervals. This analysis is built on the assumption that the oscillator pops instantaneously from one phase to another of its cycle. The same assumption led to the same equations in Ottesen⁶², Pavlidis³³, and Pittendrigh's⁶³ analysis of circadian rhythmicity (hence the acronym 'POPP' model). The success of the POPP model for circadian rhythms in its only experimental tests (using *Drosophila*⁶³, *Kalanchoe*

flowers⁶⁴ and sparrows⁶⁵) makes it worthwhile to test directly its underlying assumption that the circadian mechanism, being a simple clock, relaxation oscillator, or quickly-recovering limit-cycle mechanism, essentially pops from one point to another on its cycle when reset.

Checking this assumption would be unnecessary if the circadian oscillator were a simple clock or relaxation oscillator, which have no accessible states other than the usual cycle. The fact that rephasing curves are smooth and change abruptly at a critical stimulus duration from mean slope 1 to mean slope 0 both excludes those two models and shows that the POPP rule requires amendment for application to limit-cycle mechanisms: for if all stimuli leave the oscillator very nearly on its limit cycle, then a long stimulus, evoking type 0 resetting, can be viewed as an uninterrupted sequence of shorter stimuli; but under POPP rules, any sequence of type 1 stimuli still gives type 1 resetting. Perhaps the POPP rule must be restricted to 'strong' stimuli or to stimuli that are 'not too close together'. In measuring how strong is 'strong' or how close is 'too close', we determine how quickly the circadian oscillator recovers to its limit cycle after a disturbance. Such an experimental test of the POPP rule in *Kalanchoe* discovered no systematic deviation from its predictions for stimuli at least 2 h apart⁶⁴, though the data fit as well to a limit-cycle model with recovery time in the order of a day^{56,57}.

But in *Drosophila*, no evidence of recovery was found. In 600 experiments, a once-reset rhythm was subjected to a strong second stimulus at various times to measure the rephasing curve of a previously reset oscillator: how quickly does it return to normal, leaving only a phase shift equal to that observed in the overt behavioural rhythm? The normal phase-dependence of the phase response was found to be attenuated in proportion to the nearness of the first stimulus to the singularity, and there was no sign of return to normal during the 48 h examined by a second pulse; for such stimuli, any interval is 'too close'. No such attenuation was conspicuous following stimuli far from the singularity; for such stimuli no interval is 'too close'^{66,67}.

So the fly's clock does not resemble a quickly recovering limit cycle mechanism, either. What it does resemble is a simple harmonic oscillator—for example a pendulum—in which disruptive stimuli result in permanent, co-ordinated shifts of both phase and amplitude without effect on frequency. In fact, a mathematical metaphor of this sort fits the data from over 1,000 diverse one-pulse and two-pulse rephasing experiments on the circadian rhythm of eclosion of *Drosophila* to within almost the reproducibility of measurements (ref. 66 and A.T.W. in preparation). The theory of isochrons and phase resetting which originally predicted the singular stimulus^{50,60} cannot be rigorously applied to such mechanisms.

Organism as a clockshop

What can such a grotesque coincidence possibly signify? The thought that most tantalises me is that the individual fly's behavioural rhythm may be determined by superposition of at least two independent circadian oscillations, each possibly arising from a simple clock, relaxation oscillator, or limit cycle mechanism. (Care was taken⁶⁶ to exclude the possibility^{56,57,64,65,70} that attenuated rhythmicity arose as an artefact of phase incoherence among individually coherent pupae of the sampled populations.)

From such indications as the overt desynchronisation of circadian rhythms among the organs and cells of higher plants⁶, the splitting of activity rhythms in flies⁷¹, bees⁶, birds⁷³, rodents⁷⁴ and primates⁷⁵, some workers have speculated that the organisms more resemble a clockshop

than a clock^{70,73-80}. Populations of weakly interacting idealised oscillators have been shown to exhibit mutual synchronisation, splitting, and more complex quasi-rhythmic-idiosyncrasies resembling those observed in large multicellular organisms^{44,81}. By an adjustment of the phase relation between their mutual influence and sensitivity rhythms, they can just as easily repel each other to remain uniformly distributed around the cycle⁴⁴. Thus overt arrhythmicity in some species is not incompatible with the notion that cellular metabolism is typically organised in 24-h patterns.

Since direct control of circadian rhythms by sensitive extra-retinal photoreception is quite common, even among vertebrates²⁻⁶, there might be little need even for weak interactions among independent cellular oscillators, all cells being synchronised in parallel directly by the external light/dark cycle. Only when completely coherent does such a population exhibit the resetting characteristics typical of the individual oscillator mechanism. This may be the situation in entrainment experiments, and the deepest reason for success of the POPP model's predictions for entrainment, despite the failure of its prediction for general two-pulse interaction.

Even though a simple clock, for example, cannot show type 0 rephasing or a singularity, the summed outputs of a population of simple clocks can if its independent members are not quite synchronous when the stimulus starts. A properly timed stimulus of the right duration greatly increases the incoherence of such a population, resulting in attenuation of the collective rhythm and correspondingly attenuated rephasing curves. Both persist as though the 'amplitude', 'intensity' or 'vigour' of circadian oscillation had been reset without affecting its period, because without interactions and without much dispersion of cellular periods, the variance of cellular phases would not change. This variance of cellular phases may be the 'second and uncontrolled experimental variable' suggested above.

These matters are elaborated in more detail (A.T.W., in preparation) elsewhere: the point I wish to make for present purposes is that the unclocklike peculiarities encountered in the most thoroughly studied circadian rhythms might be accounted for in three different ways.

(1) Without invoking a multiplicity of independent oscillators, by finding a plausible mechanism with amplitude as labile as phase, and frequency nearly independent of amplitude. Predator-prey kinetics on a chemical level has this character⁸². Goodwin^{15,83} proposed a mechanism based on control of nuclear DNA-dependent RNA synthesis which could oscillate with a period of many hours in the required orbitally stable way.

(2) By supposing that whole-organism behaviour is governed by the superposition of two identical and independent smooth limit-cycle oscillations of 24-h period, perhaps one on each side of the brain^{71,72}. When their phases differ greatly, for example after both have independently recovered to random phases from near the singularity, the 24-h component of their combined outputs would be quite weak. This idea could be tested by one-sided surgical "clockectomy".

(3) By taking seriously the evidence noted above that circadian rhythmicity is an autonomous cellular process. If the cells are assumed not to interact much (as in a clockshop), then whether they are simple clocks, relaxation oscillators, or limit cycle mechanisms, the qualitative peculiarities of rephasing behaviour follow, thus harmonising phylogenetically widespread rephasing behaviour with the apparent diversity of evolutionary origins and physiological mechanisms of circadian rhythmicity.

On this alternative, the damping rate of free-running rhythms measures the range of individual cell periods and the 'clockshop' conjecture is implausible to the extent that this range is implausibly small. Persistence of rhythmicity in

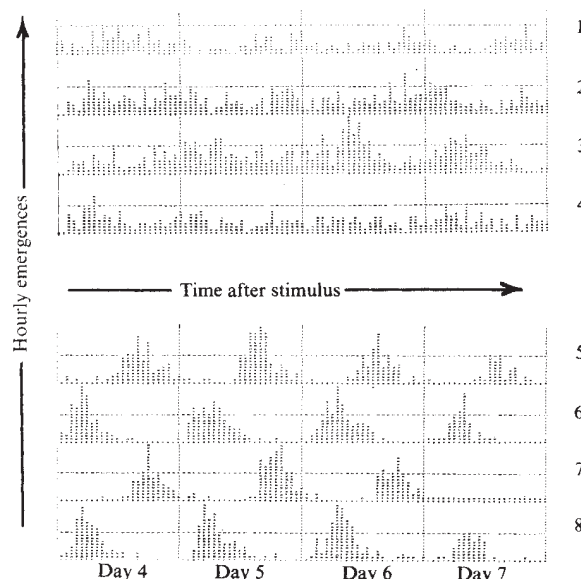


Fig. 4 The eclosion rhythm of 8 populations of *Drosophila* is recorded hourly through days 4–8 after stimulus time. Each mark in the histogram represents 1 fly. Populations 1–4 received about 5,000 erg cm⁻² of blue light near subjective midnight. Their rhythmicity is attenuated relative to populations exposed at any other combination of intensity and phase; in all such cases, for example histograms 5–8, the normal rhythm is merely phase-shifted (in Figs 2 and 3, peaks like the 15 shown here are each represented by a single dot).

Drosophila for at least 1 d without indication of damping^{28,70} suggests a dispersion of periods smaller than 2%. In *Gonyaulax* cell suspensions, rhythmicity of cell division persists unattenuated for at least 10 days⁸⁴. Assuming no mutually synchronising interactions, this also suggests that cell clocks differ in period by less than about 2%. Chemical interactions were tested for by mixing differently phased cell suspensions without revealing any tendency to synchronisation^{85–87}. The corresponding test in multicellular animals or plants would be parabiosis of individuals reared on different light cycles, or parabiosis of a rhythmic individual with one rendered arrhythmic by the singular stimulus. Discovery of strong synchronising interactions would eliminate the 'clockshop' conjecture⁸⁸. (Of course electrical interactions among nerve cells would escape detection by these means.)

Finally, this model predicts that an organism in the low-amplitude condition should exhibit a conspicuous scattering of cell phases. No such dispersion of cellular clocks states is predicted under the orbitally stable oscillator model (1). Since some cells exhibit circadian rhythms of nuclear volume, chloroplast shapes and so on⁶, this might afford a critical test of the 'clockshop' conjecture.

Either way, there remains the Darwinian question: given labile amplitude, how does it fit into the organism's life style? Has it a selective advantage or is it an 'accidental' selectively neutral by-product of something more important? My guess is that the clockshop hypothesis is correct, and that 'amplitude' lability is a laboratory artefact, the cellular incoherence necessary for its appearance being rare under field conditions of entrainment to natural photoperiods. The possibility should, however, be investigated that this 'unclocklike' feature of circadian organisation is hinting to us of some adaptive role other than the 'time' and 'phase'-oriented purposes considered up to now.

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articles

Mesozoic and Cainozoic opening of the Labrador Sea, the North Atlantic and the Bay of Biscay

Willem J. M. van der Linden

Atlantic Geoscience Centre, Geological Survey of Canada, Bedford Institute of Oceanography, Dartmouth, Nova Scotia, Canada

A reinterpretation of magnetic anomaly and basement trends suggests that the Labrador Sea opened in two stages. During a first phase, most likely in the late Jurassic–early Cretaceous, the opening was coupled, by way of a triple junction off Spain, to spreading in the central Atlantic and Bay of Biscay. A second phase of seafloor spreading in the Labrador Sea occurred between 60 and 47 Myr ago and was connected through a triple junction south of Greenland to spreading in the northern and central Atlantic.

THE Labrador Sea is thought to have formed as the result of the rifting apart of Labrador and Greenland and the formation and spreading of the sea floor in between^{1–11}. The correlation of magnetic and basement trends presented here differs from that of previous workers^{4,7,11,12} and points to an alternative geometry and spreading history for the Labrador Sea and the North Atlantic Ocean.

Magnetic trends

The residual magnetic field over the north-western Atlantic Ocean and the Labrador Sea (Fig. 1) is derived from maps in refs 4, 11, 12 and 13. Although there are inconsistencies, the overall trends and characteristics of the anomaly sequences match very well.

Over the Labrador Shelf, Precambrian rocks, covered by a wedge of seaward-thickening sediments, produce a pattern of chaotic high frequency anomalies. The zone beyond the shelf edge, which is 170 km wide and underlies the Continental Slope and Rise, is interpreted as foundered continental crust¹³. The magnetic field over this zone is characterised by small amplitude, large wavelength anomalies that run parallel to the margin. The approximate seaward limit of this zone (Fig. 1) is interpreted as the true boundary between continent and ocean.

In the Labrador deep sea, there are two sequences of oceanic anomalies with a regular and parallel pattern. Sequence I comprises all anomalies that run NW–SE, in the northern and in the southern parts of Fig. 1, although they are partially separated by a central zone of attenuated anomalies. The attenuated anomalies outline a basement high (the Cartwright Arch), which is the offshore extension of the Grenville Province¹³. Sequence II, labelled 19–24 in Fig. 1, runs almost due north from the Charlie Gibbs Fracture Zone up to about 56°30'N. There it bends westwards through approximately 80°,

and continues along this trend for about 200 km. Here the sequence changes direction again and at the same time the anomalies converge; they finally wedge out at approximately 57°N 50°30'W.

The termination of sequence II provides the key to the new correlation. The pattern is more clearly illustrated in Fig. 3a of Vogt and Avery¹¹, where the anomalies are presented with a contour interval of 50 gamma. The new correlation differs from earlier correlations^{4,7,11,12}, which, despite the pinching out near 57°N, continue sequence II into the northern part of sequence I.

The age of sequence II has been defined accurately using the time scale of Heirtzler *et al.*¹⁴. A match was obtained with anomaly sequence 19–24 which indicates that spreading occurred 47 to 60 Myr ago^{4,12}. The age of sequence I has not been accurately determined and various spreading periods have been postulated: 65–80 Myr (ref. 3), 60–80 or 82 Myr (refs 4 and 12), 60–76 Myr (ref. 5) and 38–52 Myr (ref. 7). The estimates (with one exception⁷) focus on a period, immediately prior to the generation of sequence II, in the late Cretaceous and early Tertiary. Many authors have considered an even earlier (perhaps epicontinental, perhaps rifted) Labrador Sea, in existence before the generation of anomaly sequence I (refs 2, 4, 5, 10, 11, 15, 16).

Basement trends

The basement morphology of the Labrador Sea region south-west of Greenland parallels the magnetic anomalies. In Fig. 1, I have indicated the culminations of basement as defined in refs 1, 4, 5, 9, 12, 13 and 15. The authors interpreted these basement highs as expressions of fossil mid-ocean ridges or former centres of seafloor spreading. Laughton considered the east–west trending basement structures at about 57°N to be continuous with north–westerly trending basement structures in the northern Labrador Sea. Vogt and Avery¹¹ accept this interpretation and propose the name Ran Ridge for this fossil mid-ocean ridge.

The correlation of basement trends is similar to that of the magnetic trends. Thus, the NW–SE trend of the mid-Labrador basement highs, parallel to magnetic sequence I, is probably continuous from the northern Labrador Sea to the Charlie Gibbs Fracture Zone. Also the east–west trending ridge, parallel to magnetic anomaly sequence II, probably terminates at about 51°W. In Fig. 1, the magnetic lineations associated with the

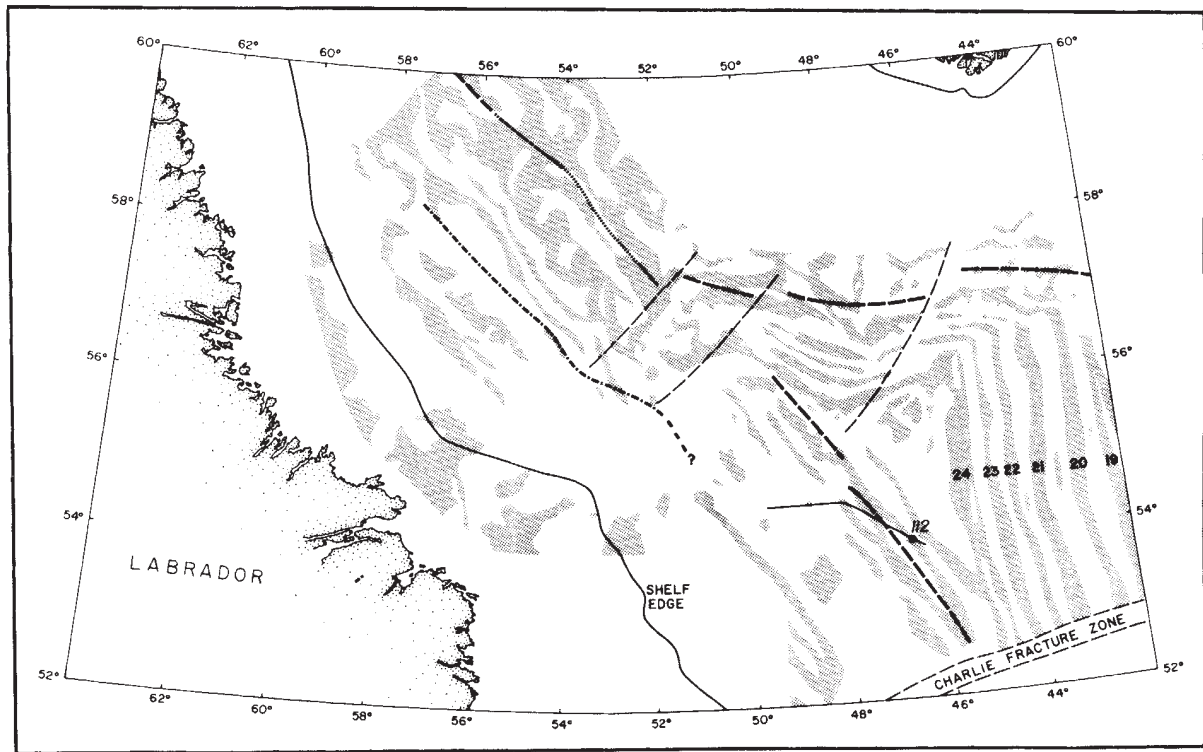


Fig. 1 Observed (— — —) and inferred (.....) positions of basement highs superimposed on the distribution of positive (shaded) and negative magnetic anomalies in the Labrador Sea (after refs 4, 5, 9, 11 and 13). — · — · — ·, The inferred continental-ocean boundary. Correlation of anomalies with the time scale of Heirtzler *et al.*¹⁴ is shown for the sequence 19–24. DSDP site 112 is marked, as well as the position of a CSS Hudson reflection profile (Fig. 5) through the site.

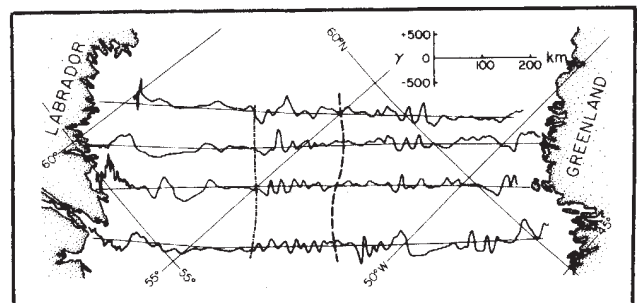
eastern half of the NE-SW spreading are only partly shown. Information from south of Greenland is too sparse and has not been included on the map. Farther north, however, aeromagnetic surveys^{7,17} indicate a mirror image of the western Labrador magnetic anomalies on the Greenland side (Fig. 2). A belt of relatively high frequency anomalies can be distinguished on both sides of a central low amplitude sequence. The centre of spreading (Fig. 2) coincides with the position of the fossil mid-ocean ridge (Fig. 1).

Labrador Sea and North Atlantic

Figure 3 combines the information for the Labrador Sea with the magnetic anomaly pattern and major structures of the North Atlantic Ocean. Sequence II (anomalies 19–24) is very well defined and can be easily correlated, particularly north of the Charlie Gibbs Fracture Zone (CGFZ)^{9,11}, where it is undisturbed by fracture zones. In contrast, the correlation of anomalies and comparison with the magnetic time scale are often ambiguous for sequence 5–18 (9–46 Myr BP), especially south of the CGFZ. The various isochrons, both north and south of the CGFZ, each identified with their age in millions of years, were taken from Pitman and Talwani¹⁸. The anomaly pattern in the Bay of Biscay and the area immediately to the west of the Bay is taken from Williams¹⁹. Vogt and Avery¹¹ indicate two basement ridges, West Thulean Rise and East Thulean Rise, about equidistant on either side of the Mid-

Atlantic Ridge, south of the CGFZ. These ridges are significant in the reconstruction of the North Atlantic, as will be shown. In the eastern Atlantic, north of the CGFZ, the magnetic anomalies run in a south-easterly direction^{4,11,12} (oblique to the 19–24 sequence to the west and parallel to the East Thulean Rise). Across the CGFZ, these anomalies do not match closely the anomalies shown by Williams¹⁹ west of the Bay of Biscay. Certainly the 81-Myr isochron through this area¹⁸ is doubtful

Fig. 2 Four aeromagnetic profiles across the northern Labrador Sea (after Hood and Bower⁷) with estimated position of centre of symmetry (— — — —) and inferred continent-ocean boundary (— · — · — · — ·). Amplitude scale in gamma.



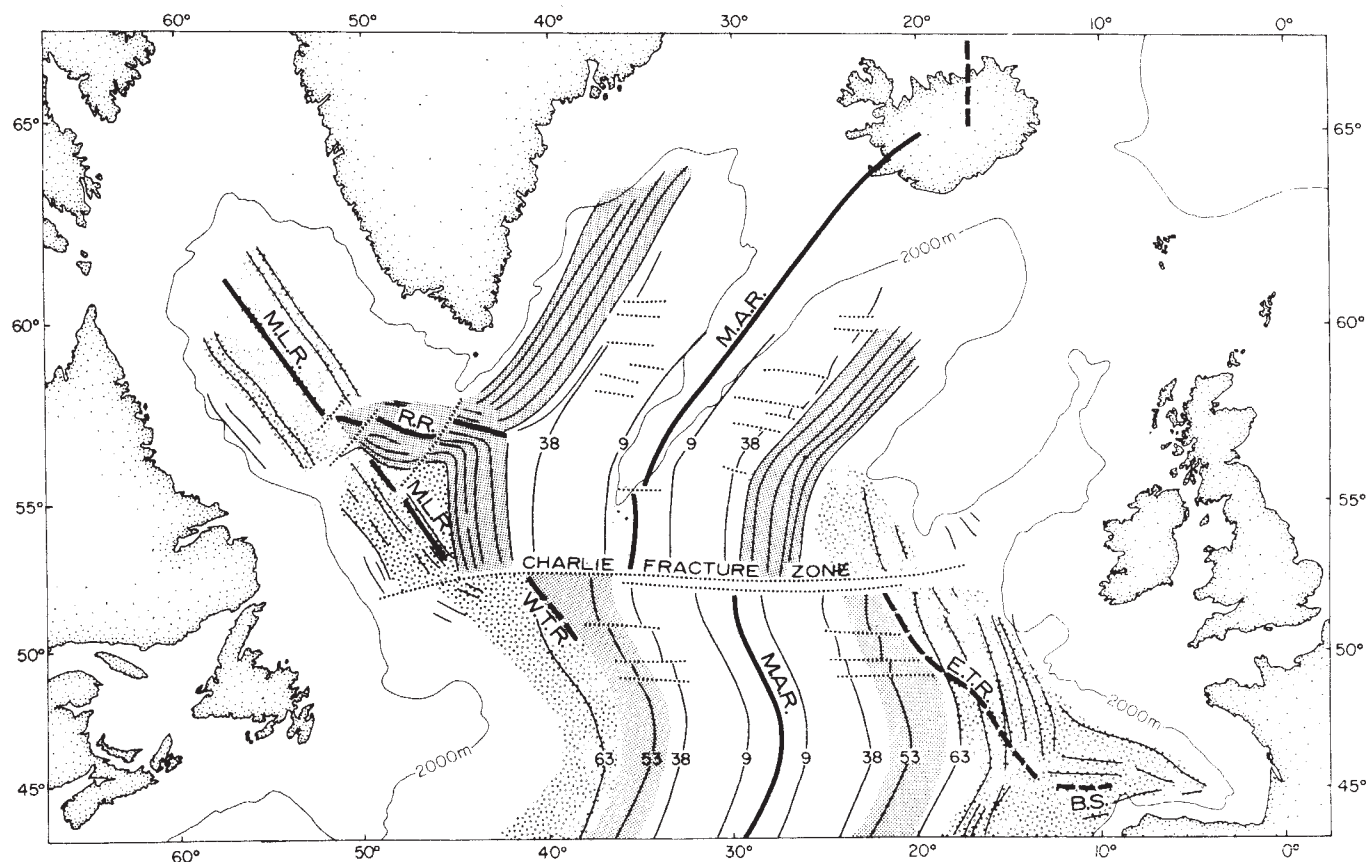


Fig. 3 Position of spreading centres (— — —), major faults (.....), magnetic anomalies and isochrons (labelled) in the North Atlantic Ocean, modified after refs 9, 11, 18 and 19. Magnetic anomaly sequence 19–24 (47–60 Myr BP) is densely shaded. Seafloor area generated during the Mesozoic is approximated with open shading. MAR, Mid-Atlantic Ridge; MLR, Mid-Labrador Ridge; WTR, West Thulean Rise; ETR, East Thulean Rise; BS, Biscay Seamounts.

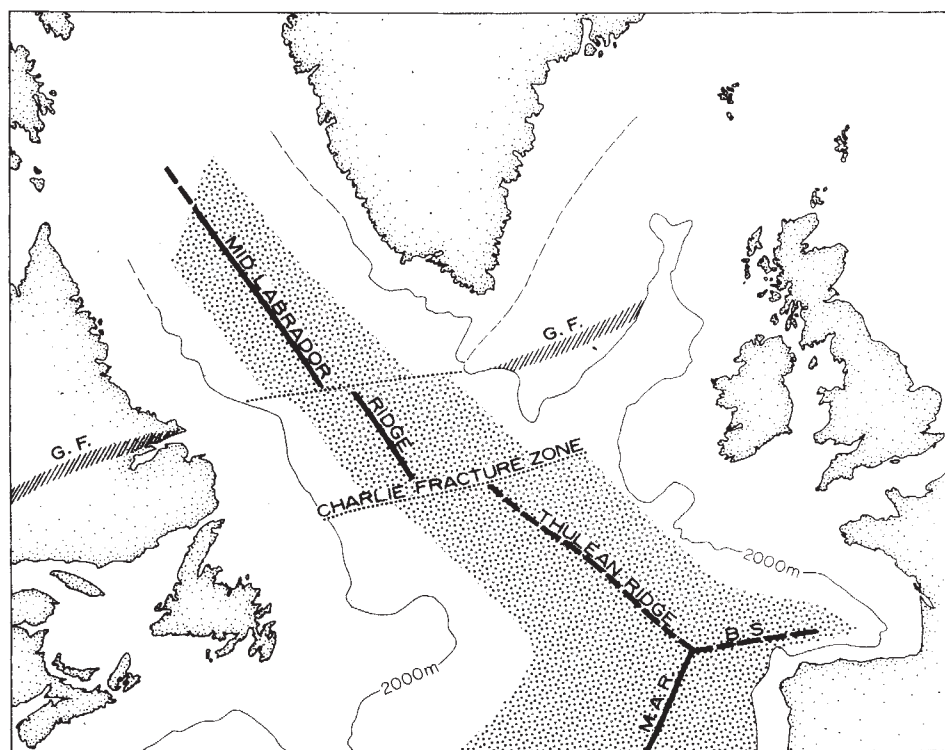


Fig. 4 The North Atlantic Ocean at the end of the Mesozoic. Seafloor area generated during the Mesozoic (phase I) is approximated with shading. The reconstruction is produced by removing the sea floor generated in the past 60 Myr (phase II and younger) and closing the gap. Note that the transform fault north of the Charlie Gibbs Fracture Zone approximates the position of the Ran Ridge. MAR, Mid-Atlantic Ridge; BS, Biscay Seamounts; GF, Grenville Front.

and is thus not included in Fig. 3. The 81-Myr isochron off the Grand Banks of Newfoundland is also omitted, for similar reasons.

I would like to retain the old name Mid-Labrador Ridge for the spreading centre, about mid-way between Labrador and Greenland, and to restrict the name Ran Ridge¹¹ to the east-west trending ridge, centred on anomaly 19.

The following reconstruction is proposed for the Labrador Sea. Phase I—the Labrador Sea opens between Greenland and Labrador with spreading centred on the Mid-Labrador Ridge. Phase II (60–47 Myr BP)—seafloor spreading is centred on the Ran Ridge and coupled through a triple junction to spreading on the Mid-Atlantic Ridge.

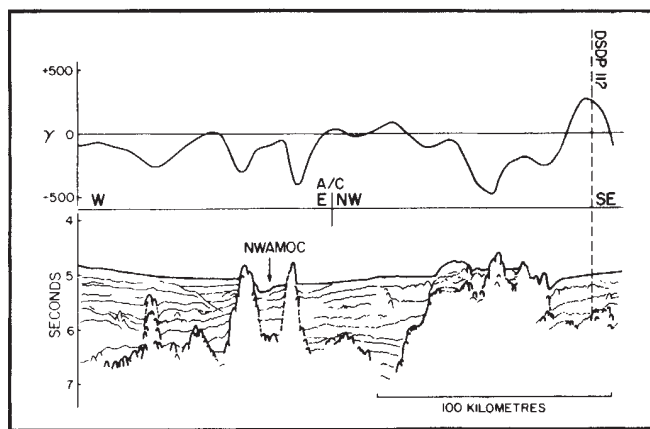


Fig. 5 Line tracing of 1,000 inch³ air gun reflection profile and associated magnetic anomalies in the Labrador Sea (CSS Hudson, cruise 72–025). Vertical scale for the reflection profile is in seconds two-way travel time. Vertical exaggeration 33:1 at water velocity. Vertical scale for the magnetic profile is in gamma. NWAMOC, North West Atlantic Mid-Ocean Channel. DSDP 112, Deep Sea Drilling Project site 112. For the position of the line see Fig. 1. Note the coincidence of the positions of negative magnetic anomalies and positive basement relief.

It is suggested that the spreading activity in the Labrador Sea during phase I was coupled to spreading in the Bay of Biscay^{15,20}. About 60 Myr ago the East and West Thulean Rises were one ridge system, as can be demonstrated by reversing the spreading in the Atlantic since that time (Fig. 4). Williams¹⁹ suggested a triple junction that tied the largely north-south directed opening of the Bay of Biscay to east-west directed seafloor spreading in the eastern Atlantic Ocean during the Mesozoic. Thus, spreading was centred on the Mid-Labrador–Thulean ridge system, Biscay Seamounts, and Mid-Atlantic Ridge. The reconstruction (Fig. 4) suggests that during phase I the Ran Ridge was a transform fault that trailed the Grenville Front¹³.

Age of spreading

The age of seafloor spreading during phase I is not firmly established but it certainly predates spreading during phase II. The shift of 25° from phase I (NE–SW spreading) to phase II (east–west spreading) (Fig. 1) constitutes a major change in the orientation of plates that most likely represents a hiatus in the spreading of the Labrador Sea.

Support for a late Cretaceous–early Tertiary age for phase I comes from Deep Sea Drilling Project (DSDP) site 112 (ref. 21). Basement samples cored at the site consisted of weathered basalt that could not be dated. A minimum age of 65 Myr was inferred for the basement from the age of the oldest sediments cored above the basalt. A seismic profile through the site taken by CSS Hudson (Fig. 5) indicates that this inference is

misleading. Eighty kilometres to the north-west the thickness of the sediments above the basement is twice the thickness found at site 112, which suggests a minimum age of the basement of 130 Myr. If a contemporaneous opening of the Labrador Sea and the Bay of Biscay is accepted then the identification of magnetic anomalies 31–32 in the Bay¹⁹ supports a late Cretaceous age for episode I. An earlier interpretation of the same anomaly pattern²², however, suggests a late Cretaceous age for the Bay of Biscay.

There are other arguments in support of an earlier, possibly Jurassic phase of opening of both the Labrador Sea and the Bay of Biscay. On the basis of geological and geophysical evidence from both Spain and France (stable Europe), the Bay of Biscay is considered to have been formed between the Triassic and late Cretaceous²³. Results from DSDP sites 118 and 119 in the Bay of Biscay suggested to Laughton¹² that the oldest sediments below the deepest dated horizon in the Bay (Palaeocene) may be as old as early Cretaceous. Palaeomagnetic results suggest an opening of the Bay of Biscay some time between the Triassic and late Cretaceous²⁴. Larson and Pitman²⁵ elaborated on the interpretation of Williams and McKenzie²² and suggested that the Biscay anomalies may be part of the Keathley sequence and thus that the Bay opened between 150 and 110 Myr BP. Correlation of the Labrador Sea and Keathley anomalies is indeed at least as good (or as bad) as the correlations between the Labrador anomalies and the Cretaceous magnetic timescale. The position of the Keathley anomalies in the central Atlantic^{18,26} would then identify the position of the third or southern Middle Mesozoic spreading arm, radiating from the Williams¹⁹ triple junction.

As mentioned, several authors favour partial opening of the Labrador Sea during Jurassic time, although no one has suggested a Jurassic age for the northern Labrador magnetic anomalies. McMillan^{10,16} argued for a Mesozoic initiation of the Labrador Sea to explain the occurrence of Jurassic carbonates collected from the Labrador continental slope.

Further support for late Jurassic–early Cretaceous rifting of the Labrador Sea comes from a series of dikes that run parallel to the coast in south-west Greenland²⁷; these have been dated as late Jurassic (138 Myr). Dike intrusion may, however, have predated actual spreading by several million years.

From the available evidence, I am inclined to accept a late Jurassic age as the most plausible for the opening of the Labrador Sea and the Bay of Biscay. A more definitive answer to the problem will have to come from more closely spaced magnetic surveys and from dated oceanic basalts in crucial areas.

I propose that (1) the Labrador Sea was formed during two phases of seafloor spreading, one in the late Jurassic–early Cretaceous and one in the early Tertiary, and (2) the Labrador Sea opening in the Mesozoic was coupled with seafloor spreading in the North Atlantic Ocean and the Bay of Biscay.

As an alternative to earlier interpretations^{4,5,12}, this model simplifies the geometry of the Atlantic evolution. A Jurassic age, suggested for the early phase of opening, is in better agreement with geological observations on the Labrador Sea margin and the Bay of Biscay. It also suggests that Mesozoic seafloor spreading in the Atlantic Ocean was not restricted to the area south of the Azores–Gibraltar Fracture Zone¹⁸.

There are several areas which have to be examined if the model is to be refined—notably, between the East Thulean Rise and Europe, between the West Thulean Rise and the Grand Banks, and between Greenland and the Ran Ridge. These areas are all near continental margins and are probably structurally complex.

Drs L. H. King and R. K. H. Falconer reviewed the manuscript.

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Nucleic acid-mutagen interactions: crystal structure of adenylyl-3',5'-uridine plus 9-aminoacridine

Nadrian C. Seeman, Roberta O. Day* & Alexander Rich

Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Adenylyl-3',5'-uridine and 9-aminoacridine cocrystallise in a heavily hydrated monoclinic lattice. The adenine residues are hydrogen bonded with uracil residues and the base pairs are stacked parallel to each other in the crystal with 9-aminoacridine sandwiched directly between them.

IN 1961 Lerman¹ suggested that planar aromatic aminoacridine molecules can be inserted between the base pairs in DNA. This mode of molecular interaction has been widely accepted and many interactions between nucleic acids and planar molecules have been explained in terms of an intercalation model. A number of varied molecules including carcinogens and mutagens have a mode of action which is believed to be intimately associated with the intercalation mechanism. In spite of a great deal of chemical and biological work, however, the detailed structure of intercalation complexes has not been determined. In part, this reflects the fact that it is difficult to obtain structural information at atomic resolution from X-ray diffraction studies of polymeric nucleic acids combined with intercalating agents. In view of the large number of important biological phenomena associated with intercalation, it is desirable to understand the detailed interactions of planar intercalating molecules with hydrogen bonded base pairs. Here we report the crystal structure of complex containing the dinucleoside phosphate adenylyl-3',5'-uridine (ApU) and the mutagen 9-aminoacridine. In the heavily hydrated crystal lattice the adenine and the uracil residues are hydrogen bonded while the 9-aminoacridine molecules are sandwiched between the base pairs and stacked parallel to them.

Experimental details and solution of structure

A solution of 5 mg of ApU (Sigma) in 0.25 ml of a 200 mM sodium cacodylate buffer at pH 6.6 was prepared. A 4.16 mg sample of 9-aminoacridine hydrochloride was dissolved in this solution, followed by the addition of 0.1 ml of isopropanol. The vial was sealed, and crystals suitable for diffraction analysis appeared within a week. These crystals were unstable in the

absence of the mother liquor and had to be mounted in a sealed capillary. The lattice constants were determined and intensity data were collected on a Syntex P1 diffractometer. The crystal data are $a=13.261$ Å, $b=26.677$ Å, $c=6.922$ Å, $\beta=95.08^\circ$, space group $P2_1$. Intensity data were collected for 3,553 Friedel pairs using nickel filtered Cu K α radiation. 2,874 reflections were observed on the basis of a 2σ significance criterion. Lorentz and polarisation corrections were applied to the intensity data, but no absorption correction has been calculated.

The standard method for solving crystal structures of dinucleoside phosphates has been to locate the phosphorus atom by means of resolution difference techniques²⁻⁴. Many attempts to apply this technique to this structure were unsuccessful because the phosphorus-phosphorus vector was hidden under a large peak on the Harker section. We were therefore forced to locate the phosphorus atom by the use of the anomalous dispersion data available from the phosphorus which has 0.43 anomalous electrons. Once the phosphorus was located, the rest of the structure was readily obtained in a series of seven Fourier cycles and the water structure was determined in a series of difference Fourier calculations. The crystal structure has been refined using least squares and anisotropic thermal parameters to a conventional R factor of 7%. The disorder in the water structure has made it impossible to locate hydrogen atoms.

Description of the structure

The molecular structure and numbering for ApU and 9-aminoacridine are shown in Fig. 1. Figure 2 shows a view of the complex perpendicular to the plane of the adenine-uracil base pair and the 9-aminoacridine molecule. In Fig. 2 the monoclinic b axis is in a horizontal orientation and the twofold screw relationship between successive molecules is clearly shown in the diagram. The thickness of the unit cell perpendicular to the plane in Fig. 2 is 6.9 Å which is twice the thickness of the planar aromatic rings. It can be seen that the adenine-uracil residues are hydrogen bonded in pairs using the nitrogen 6 of adenine bonding to oxygen 4 of uracil (hydrogen bond length = 2.99 Å) and nitrogen 3 of uracil bonding to nitrogen 7 of adenine (2.84 Å). This hydrogen bonding arrangement, first observed by Hoogsteen⁵, has been seen in a large

*Also at Department of Chemistry, University of Nebraska, Lincoln, Nebraska 68508.

number of purine-pyrimidine complexes⁶. The hydrogen bonded base pair is surrounded on both sides by 9-aminoacridine molecules as shown in Figs 2-4. In addition to this, the adenine residues are hydrogen bonded to the ribose hydroxyl groups of an adjoining uridine where the interactions are between the N6 amino group of adenine and O3' of the ribose (2.95 Å) and between N1 of adenine and O2' of the same uridine ribose (2.77 Å). Although the hydrogen bonding to uracil involving the adenine imidazole N7 nitrogen has been seen in many complexes, the hydrogen bonding between adenine and ribose in this specific manner has not been seen previously.

The projection in Fig. 2 shows an unusual circular cylindrical channel, the walls of which are composed of four different molecules of ApU and three molecules of 9-aminoacridine. The entire structure is built out of clusters of these cylindrical complexes, one complete unit of which is shown in Fig. 2. The interior of the cylinder is heavily hydrated containing 15 water molecules which are disordered among 21 loci in the asymmetric unit. Since these hydrated channels go through the entire crystal, it is likely that this gives rise to the instability of the crystal on drying.

Figure 3 shows a view of one segment of the wall of the cylindrical channel viewed perpendicular to the direction shown in Fig. 2. Here the insertion of the planar 9-aminoacridine molecules is shown between the adenine-uracil hydrogen bonded pairs. The adenine-uracil pairs are nearly coplanar, but have a slight propeller twist of 4° between them.

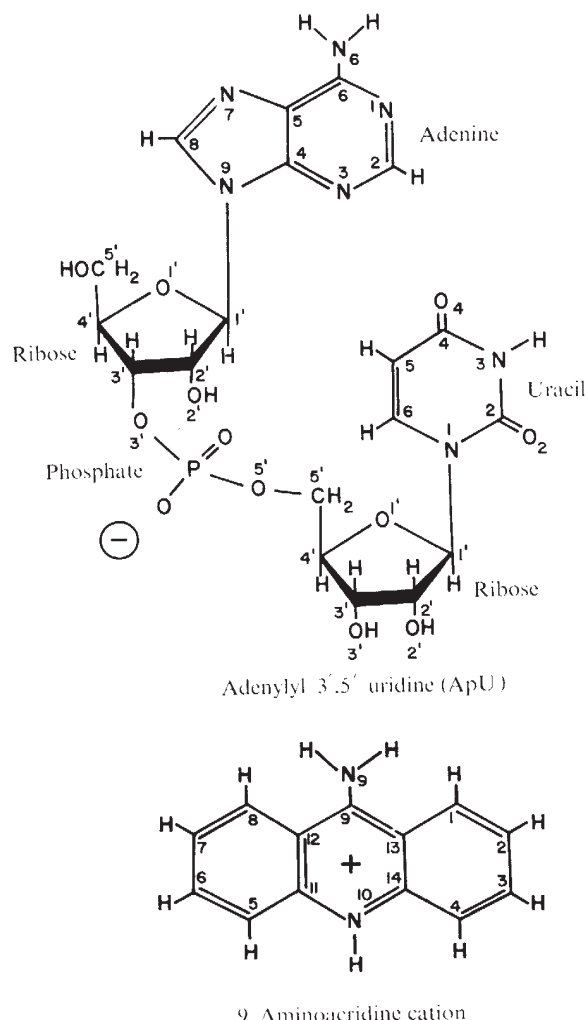


Fig. 1 The chemical formulae and molecular numbering system for adenylyl-3',5'-uridine and 9-aminoacridine.

Nucleoside conformations are unusual

The perturbation of the dinucleoside phosphate structure by the mutagen results in some unusual features for the nucleosides. The adenosine ribose has a C2' endo conformation with a glycosidic torsion angle of 77° which is in a region between the syn and anti conformations^{7,8}. The glycosidic torsion angle is not unusual for nucleosides which have a C2' endo ribose; however, this is the first time that a C2' endo conformation has been seen in a dinucleoside ribophosphate. The ribose of the uridine nucleoside is in the more familiar C3' endo conformation; however, the glycosidic torsion angle is again

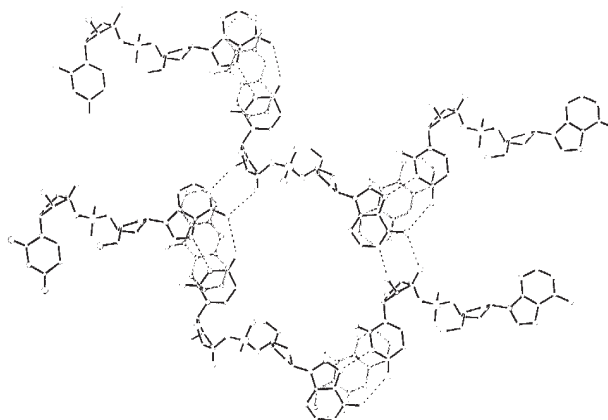


Fig. 2 A view of the structure perpendicular to the planes of the bases and 9-aminoacridine. The 9-aminoacridine does not have darkened bonds. The dashed lines indicate the hydrogen bonding interactions of adenine. The *b* axis is horizontal, and the cylindrical water channel is seen here.

rather unusual having a value of 71° which has not been reported previously for the 3' endo sugars. As can be seen in Fig. 3, the bases are approximately parallel to the ribose O1'-C4' bonds rather than O1'-C1' as is usually the case^{8,9}. It is interesting that the puckering of the adenosine ribose ring and the values of both glycosidic torsion angles are characteristic features of deoxyribonucleotides rather than ribonucleotides¹⁰.

The backbone connecting the two nucleosides is in a helix-reversing A1 conformation⁹ which was first seen in the crystal structure of UpA². All the bond angles and distances in the dinucleoside phosphate are in the normal range; the only unusual features of the structure associated with the perturbation caused by the acridine are the unusual glycosidic torsion angles and the conformation of the adenosine ribose ring. In contrast to this the torsion angles of the backbone are comparable with those which have been observed in all previous dinucleoside phosphate structures^{2-4,11}. It is interesting to ask what would happen if one inserted the adenosine and uridine nucleosides observed here into a double helical structure such as those found for the sodium salts of ApU³ and GpC⁴. The result would be a structure with an increased distance between the adjacent bases along the chain. The normal 3.4 Å stacking distance would be approximately doubled, allowing the acridine to be intercalated between the base pair.

A view of the hydrophobic intercalated column containing the acridines and the base pair is shown in Fig. 4 where we are viewing the column at an angle which allows us to visualise both the base pair and the acridine atoms. The unusual glycosidic torsion angles can be seen in this projection as well as in the projection shown in Fig. 3.

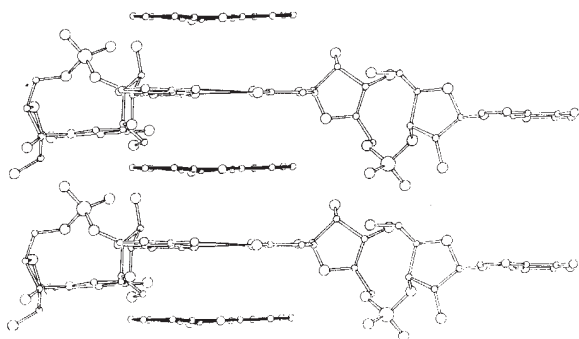


Fig. 3 A view of the intercalative structure perpendicular to the view in Fig. 2. The bases and 9-aminoacridine are viewed end on. The acridine molecules have darkened bonds.

Stacking interactions

Water molecules are excluded from the hydrophobic column shown in Figs 3 and 4, and this undoubtedly contributes to its stability. The stacking interactions are very great in this complex and these no doubt provide the driving force for forming the complex. The water excluded from these areas is found in the elongated channels. The detailed nature of the water structure will be described elsewhere.

Another feature of the present structure is the fact that electrostatic interactions probably play only a small role in stabilising the structure. The acridine molecule is charged and, as shown by Talacki *et al.*¹², part of the positive formal charge resides on the amino group and part is on N10. These atoms are both more than 6 Å away from the nearest phosphorous atom. Because of this considerable distance electrostatic interactions are likely to be far less important in stabilising the structure than the considerable stacking interactions. It is interesting that the acridine C9-N9 bond is antiparallel to and almost overlapping the adenine C8-N7 bond. This and other overlaps seen in Fig. 2 suggest that the fine details of the stacking may be stabilised by small local electrostatic interactions.

The detailed nature of the interaction of the 9-aminoacridine with the base pair is of interest. Figure 2 shows that each of the two outer six-membered rings of the acridine is largely overlaid by a purine or a pyrimidine. The centre ring is largely found in the region occupied by the hydrogen bonding interactions. The relationship of the stacking interactions seen with this hydrogen bonding to the interactions which might be found in a double helix involving the normal Watson-Crick hydrogen bonding is not entirely clear. One possibility, however, is that the interaction of the 9-aminoacridine with either the uracil or the adenine might be preserved in a double helix. If the uracil-acridine interaction were conserved with a normal Watson-Crick base pair, heavy overlap with the adenine would result. Alternatively, if the overlap with the adenine residue were maintained, there would be no overlap between acridine and the uracil. However, in this latter position the 9-amino group of the cationic mutagen would be in a position to form strong hydrogen bonds with the anionic phosphate group. It is interesting that two different binding modes have been described for intercalating agents¹³.

Features of this mutagen-dinucleoside phosphate complex might have relevance in other structures. It is known that planar intercalative molecules can combine with transfer RNA¹⁴. There are two hydrogen bonding interactions in yeast phenylalanine tRNA which involve uracil derivatives hydrogen bonding to imidazole N7 of adenine^{15,16}. It is conceivable that the interaction of planar intercalating molecules may interact at these sites in a manner similar to that seen in this structure. Another interesting feature of the present

structure is the unusual hydrogen bonding found between the ribose residue and the adenine ring. This type of interaction might be specific for adenine and it might be associated with some of the constant adenine residues which are found in tRNA where they may hydrogen bond to ribose residues.

This is the first molecular structure analysis carried out at atomic resolution which clearly indicates the mode of intercalation of a mutagen between an adenine-uracil base pair. The structure has some unusual features, some of which are suggestive of the manner in which planar molecules may be intercalated into double helical structures or into other more complex nucleic acid structures such as tRNA or ribosomes. This structure illustrates one stable mode of interaction between a mutagen and a base pair. Aspects of this structure may be of help in providing a detailed understanding of the mode of action of mutagens in biological systems.

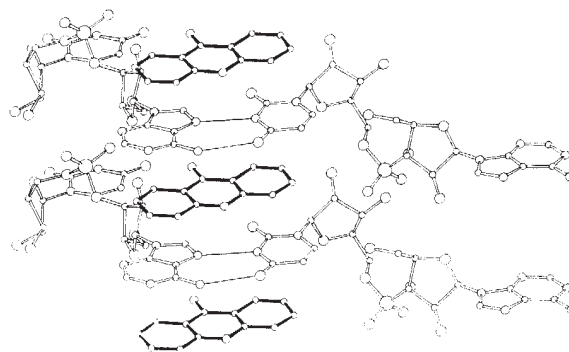


Fig. 4 A view of the intercalative stacking interaction at an oblique angle. The nature of the vertical hydrophobic column of alternating base pairs and 9-aminoacridine is seen here.

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Note added in proof: We were recently sent a preprint of a paper by C. C. Tsai, S. C. Jain and H. M. Sobell describing the crystal structure of an ethidium-dinucleoside phosphate complex in which ethidium is intercalated in a fragment of RNA double helix¹⁷. The nucleosides show some of the same perturbations described here. We thank Dr Sobell for providing us with this information.

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letters to nature

Jupiter's main magnetic field measured by Pioneer 11

THE first *in situ* studies of the magnetosphere of Jupiter were conducted in November and December, 1973, by the spacecraft Pioneer 10. An analysis of the helium magnetometer data¹ indicated that the planetary magnetic field was well represented by an offset tilted dipole (OTD) at distances less than $10R_J$. (R_J =radius of Jupiter=71,372 km). But the movement ($4.0 \text{ gauss } R_J^3$) and tilt (10.6° at $\lambda_{III}=$

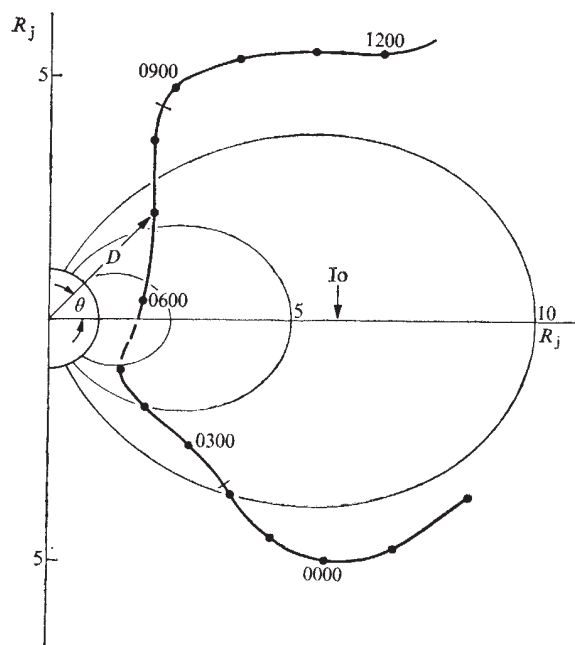


Fig. 1 Path of Pioneer 11 spacecraft as function of magnetic latitude, θ , and distance, D , near closest approach to Jupiter, which occurred at 0522 GMT December 3, 1974. Numbers on path indicate time (GMT). Location of orbit of satellite Io is indicated.

222°) of this model, identified at D_2 , yielded a field configuration and intensity which were inconsistent with several earlier independently derived estimates obtained from ground based observations of radio emissions²⁻⁶.

A second opportunity to study the main magnetic field of Jupiter occurred on December 3, 1974, when Pioneer 11 passed within $0.6 R_J$ of the planetary surface. The near planet trajectory is shown in Fig. 1, presented in a magnetic latitude and distance format using the assumed D_2 model field⁷. The wide excursion in latitude is accompanied by a similarly wide coverage in longitude, extending 660° from $\lambda_{III}=30^\circ$ at 0000 GMT to $\lambda_{III}=330^\circ$ at 1100 GMT.

A high field magnetometer was placed on Pioneer 11 by NASA-GSFC to provide extended range coverage up to 17 gauss, values believed representative of high latitude, low altitude field intensities from radio astronomy observations²⁻⁶. Here we present the preliminary results of an analysis of quick-look data obtained from this instrument. We find that within $3 R_J$, the planetary magnetic field is much too complex to be represented by a simple OTD and that higher harmonic multipoles are required.

The instrumentation aboard Pioneer 11 consisted of a single range triaxial fluxgate magnetometer capable of measuring fields up to 10 gauss along each orthogonal axis, and associated electronics. The quantisation step size for the 10-bit A/D converters used is ± 600 gamma for fields less than 2 gauss. An instantaneous vector measurement of the magnetic field is obtained once every three revolutions of the spacecraft (36 s) when a reference axis crosses through the ecliptic. The magnetometer weighs 272 g and uses 0.3 W of power from the GSFC Cosmic Ray Telescope Experiment. A more complete description is given in ref. 8.

The raw magnetic field data are translated to a Jupiter centred spherical coordinate system and combined with spacecraft trajectory information to yield a measurement

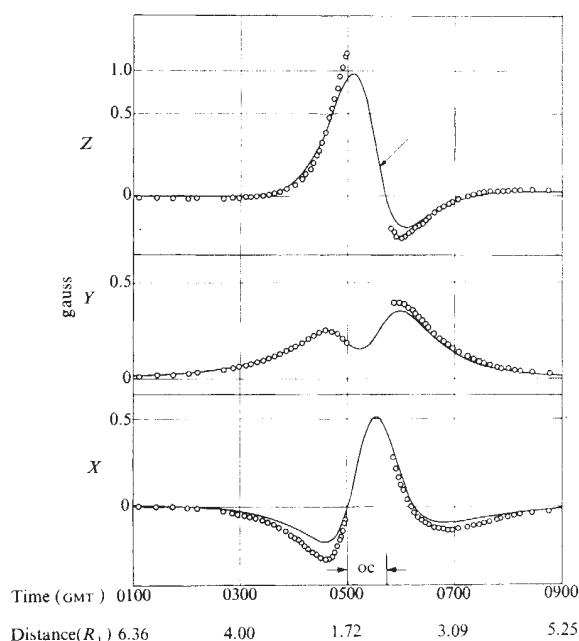


Fig. 2 Comparison of expected magnetic field of Jupiter (solid line) assuming simple offset tilted dipole magnetic field model with observations (circles) by GSFC high field triaxial fluxgate magnetometer. Period of occultation of radio transmission of data indicated by OC. Spacecraft-centred inertial cartesian coordinates are used (z axis points to Sun, x axis points to south ecliptic pole).

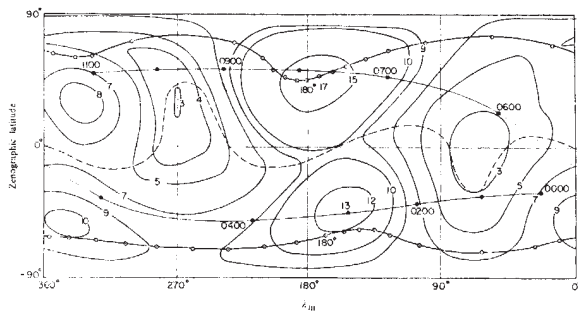


Fig. 3 Isointensity contour map of magnetic field on surface of Jupiter predicted by GSFC model O₁, using 15 spherical harmonic coefficients up to octupole moment. Field strength of each contour given in gauss. Trace of sub-Pioneer 11 point, at time (GMT) on 3 December, is indicated accordingly (four digit numbers). For Io footprint, equal 20° longitude increments at Jovigraphic latitude = 0° and radial distance = 5.95R_J were used as starting points. Note significant distortion of field lines from meridian planes. —, Footprint of field line through Io; ---, sub-Pioneer 11 trace; ···, magnetic equator.

set. Conversely, given a magnetic field model, we can obtain the equivalent readings which should be observed in each orthogonal axis of the instrument coordinate system. This is illustrated in Fig. 2 where the theoretical digital counts expected from the D₂ model are plotted along the Pioneer 11 trajectory (solid line) and compared to the observations (circles). For radial distances greater than 3R_J, there is reasonable agreement with the predicted values but closer to the planet, the field magnitudes increase rapidly, much faster than the expected inverse cube law for a dipole. This implies the existence of higher order multipoles, which become very significant for distances smaller than 3R_J.

The observations between 1.7 and 5.6R_J have been fitted in a least squares sense to first, second and third degree spherical harmonic expansions—terms corresponding to a centred dipole, quadrupole and octupole moments. We find a significant decrease in the root mean square (r.m.s.) of the residuals when quadrupole and octupole terms are included. An offset tilted dipole representation, which uses six of the eight dipole plus quadrupole coefficients, yields a vector r.m.s. of 0.026 gauss which is considerably larger than the quantisation step size of the instrument; whereas an octupole expansion, using 15 coefficients, reduces the r.m.s. value to 0.019 gauss. This is a clear indication that the main field of Jupiter is much too complex to be represented by an OTD, especially for $R < 3R_J$.

To illustrate the complexity of the field topology near the planet, the surface field derived from the 15 coefficient spherical harmonic expansion, that is, terms up to and including an octupole, has been plotted in the form of isointensity contours as shown in Fig. 3. The field strength at the poles is asymmetrical and much larger, 17 and 13 gauss in the north and south respectively, than that obtained from OTD representations. The magnetic equator, as defined by the zero dip angle, is highly distorted with respect to the Zenographic equator. Some caution must be exercised in the interpretation of these results since they represent only one solution of many possible fits to the observations along the spacecraft trajectory. This means that inclusion of higher order multipoles and/or a different data interval lead to a modified coefficient set. Nevertheless, the contribution of higher order multiple terms is evident, independent of the particular expansion used to derive the surface field. Thus, the differing results obtained in separate studies of the radio

emissions may be capable of being resolved in terms of different source locations and the sensitivity of the interpretation to distortion by non-dipole terms.

The isointensity surface map in Fig. 3 shows many unique features. Most remarkably, the 'footprint' of the field line through the satellite Io passes near both the north and south magnetic dipole regions. So particles mirroring on the L shell of Io also mirror in the Zenovian auroral regions. The hemispherically asymmetrical field leads to uniquely asymmetrical mirror points for such trapped particles. The field intensities in the polar regions are now high enough to allow further elaboration and detailed study of the relationship of decametre emissions by Jupiter and their modulation by Io²⁻⁶. The longitude of the north polar region, $205 \pm 15^\circ$, is quite consistent with much prior work²⁻⁶. These four features suggest to us that the source of decametre radio emission from Jupiter is sporadic precipitation of particles into the Zenovian auroral regions. Although the distortion of the magnetic equator is large at the surface, we find that it rapidly approaches a simple OTD model at $R > 2.5R_J$. The dipole terms of the OTD correspond to a dipole moment of 4.2 ± 0.1 gauss R_J³ at a tilt angle of $8.5 \pm 1.0^\circ$ and a longitude = $225^\circ \pm 10^\circ$.

The motion of charged particles in such a non-axially symmetric field configuration means that a further complexity is added to the interpretation of such observations. Indeed, it is these asymmetries which also suggest to us that the reason for the 10-h periodicity^{9-12,13} in charged particle observations on Pioneer 10 depends in part on a special azimuthally asymmetric magnetospheric configuration. Thus, a particular field geometry occurs only once every Jovian rotation, rather than the twice which is demanded by any nearly axially symmetrical model such as an OTD with small offset.

We believe that these results and the derived quantitative octupole model of the main magnetic field of Jupiter will permit reconciliation of the previous 20 yr of radio astronomy data with the real magnetic field of Jupiter. Moreover, with the *in situ* charged particle observations by Pioneer 10 and 11, an understanding of the basic source mechanisms and processes leading to the suite of emission phenomena should now be possible.

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M. H. ACUNA
N. F. NESS

Laboratory for Extraterrestrial Physics,
NASA-Goddard Space Flight Center,
Greenbelt, Maryland 20771

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New microstructure of decametre solar radio bursts

THE fine structure of a variety of decametre solar radio bursts obtained with high temporal and frequency resolution spectrographs provides valuable information on the generation mechanisms of these bursts together with the physical conditions in the corona. Earlier observations¹⁻¹⁰ of solar radio bursts in the decametre region have revealed some types of fine structure bursts, drift pairs and split pairs being examples of fine temporal and frequency structure bursts respectively.

We report here a new type of solar decametre bursts which, we believe, has been seen for the first time. This was a result of high resolution (frequency ~ 5 kHz and time ~ 10 ms) spectral observations made over a frequency range of only 0.5 MHz near 35 MHz with a scanning rate of 100 Hz. A circularly polarised crossed-Yagi antenna with an effective gain of about 8 db was used to track the Sun. Recording was on a continuously moving 35-mm film.

This high resolution spectrograph, went into regular operation in February, 1974. Since then a large number of solar radio bursts, including a variety of fast drift bursts (individual, in large groups, as well as storm bursts) have been recorded. Three noise storm periods, one each in July, August and September, 1974, were interesting.

Figure 1a and b shows examples of a new class of bursts which occurred respectively on July 17 and August 6, 1974. Each of the bursts, which we call 'complementary bursts' is characterised by two components, the first of which is essentially a fast drift burst comprising patches of strong and weak emissions (or emission gaps). After a time delay of ~ 0.5 s, narrow bands of intense emission are seen to have occurred. They cover a frequency range of ~ 30 –70 kHz which is almost equal to the one corresponding to the emission gap in the first component. Figure 1c shows another event which occurred on July 16, 1974. Here the intense emission in the second component covered a range of ~ 350 kHz against the very weak emission in the first component. Again, the time delay between the two components was ~ 10 s.

Figure 1d shows another example of this type of burst. This event was recorded on September 13, 1974. It is interesting that there are three components in this burst. Furthermore, in this case there is an intense narrow band emission in the second component corresponding to the emission gap in the first component; and corresponding to the emission gap in the second component there is an intense narrow band emission in the third component. These examples show that, in general, the frequency drift rates of these narrow band emissions are less than those of their first components. In particular, the drift rates are higher if the intercomponent time delay is more (Fig. 1c).

Thirty four 'complementary bursts' were recorded from July through September, 1974. On average, two different groups of delays between the components of bursts are observed. The duration, at a given frequency, of all the components is ≤ 1 s. Figure 2 is a histogram of the time delay between the components of bursts and the number of bursts. It shows two delay groups centred around 1–2 s and 10–15 s. The emission gap for the first group covers a frequency range of about 30–70 kHz. For the second group it covers a range of about 100–200 kHz. Again, the delayed narrow band emissions of the bursts show frequency drift rates of ≤ 500 kHz s⁻¹ for the bursts with the intercomponent delays of 1–2 s and ≥ 1 MHz s⁻¹ for the bursts with 10–15 s intercomponent delays.

The phenomenon described here could possibly be the result of either the same stream of electrons, or to two or three successive electron streams. In the case of more than one stream, the first stream, while moving out through a smooth

coronal plasma in which the conversion of plasma waves into transverse electromagnetic waves is very poor, might excite only sporadic emissions. Such a stream might create some turbulence behind it. The next stream, which encounters such a turbulence, should give rise to a continuous type III emission which is not what is observed. It is thus difficult to imagine how the intense narrow band emissions in the later components could occur only over the frequency range corresponding to which there are emission gaps in the previous component. The possibility of successive streams was invoked to explain type IIIb associated with type III bursts⁶.

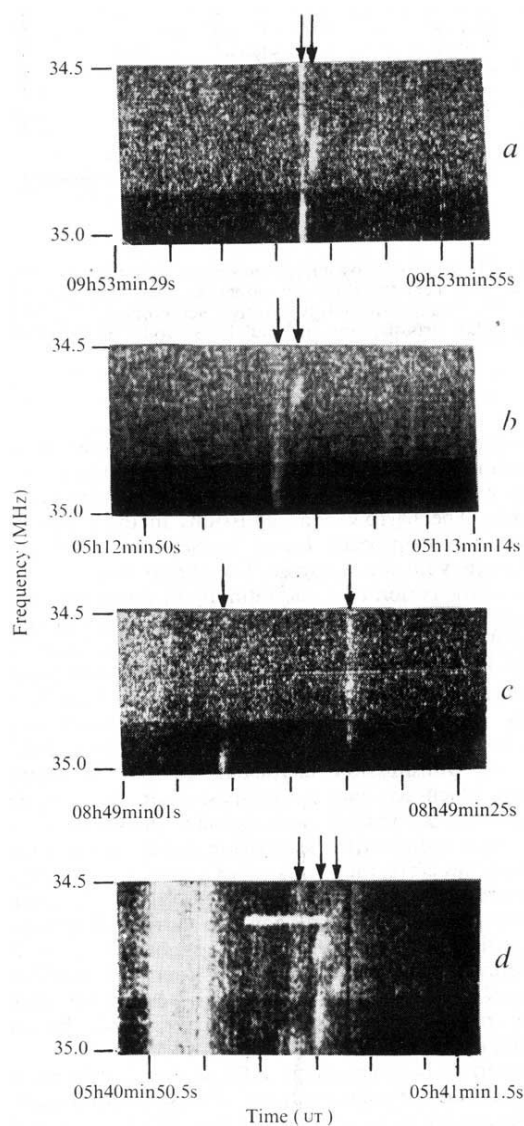


Fig. 1 Dynamic spectra of solar bursts near 35 MHz. *a*, Burst on July 17, 1974 showing two components (marked by arrows above), the first with an emission gap around its centre, the second strong and delayed by ~ 0.5 s. *b*, Similar event on August 6, 1974. *c*, Burst on July 16, 1974; the second bright component is delayed by ~ 10 s. *d*, Bursts on September 13, 1974. The bright vertical patch is a group of type III bursts. The next event shows three components displaying similar characteristics as the two-component events. Horizontal bright line is due to a local transmission. The lower dark strip in each photograph is due to receiver gain variation of ~ 1 db.

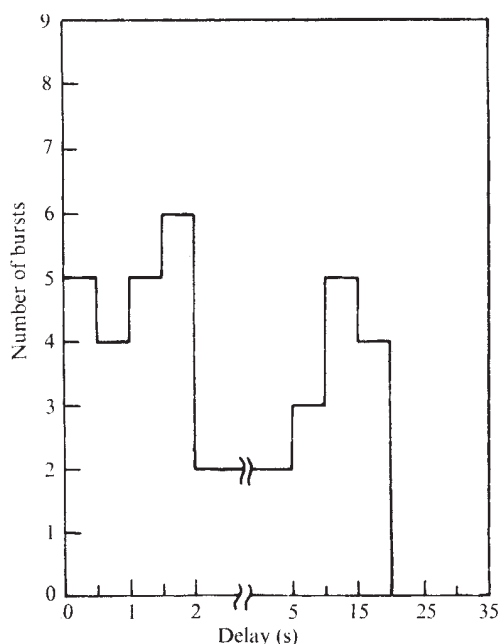


Fig. 2 Histogram showing the number of bursts as a function of time delay between their components. Total number of bursts $n = 34$ observed from July through September, 1974. Note the two delay groups, one around 1–2 s and the other around 10–15 s.

On the other hand, if a single stream model is assumed the sporadic emissions of the first component may be due to the sporadic leakage of electrons moving along a coronal streamer. The narrow band emissions in the following components may then occur due to the electrons which propagate at an angle with the streamer. The region where this happens might be the region of a discontinuity at the interaction of the streamer with another coronal structure moving with a different velocity¹¹. The observed slower frequency drift rates of these narrow band emissions then depend on the angle between the direction of propagation of these electrons and the plasma density gradient in these magnetically turbulent regions¹². It is also possible that the narrow band emissions could be the result of transmission characteristics of the propagation medium which acts like a band-pass filter. But this does not explain why the narrow band emission of the second component always corresponds with the emission gap in the first component. We must, therefore, think in terms of duct-like coronal structures which trap and delay part of the electromagnetic radiation of the first component, analogous to terrestrial whistler mode propagation. White light coronagraphs of coronal loop structures lasting for less than an hour and observed over distances from 1.5 to 6 solar radii were made during the Skylab mission¹³. These structures may provide the necessary trapping ducts.

The narrow band emissions in the second component of these bursts are relatively more intense. If these emissions are due to the acceleration of electrons in the coronal structure interacting with the coronal streamer, then one should invoke a process whereby the amplitude of the intensity of these emissions is built up.

We note that these complementary bursts are a distinct class by themselves in the sense that there is no association between them and type III bursts. Clearly this is different from the association between type IIb and type III bursts wherein the former occurs as a precursor to the latter⁶.

The frequency structures that have been reported here as complementary bursts cannot be attributed to the effect of Faraday rotation since our antenna is circularly polarised.

Polarisation observations of this type of bursts would be rewarding. So a provision is being made in the spectrograph for making observations of right and left circular polarisation.

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H. S. SAWANT
S. K. ALURKAR
R. V. BHONSLE

Physical Research Laboratory,
Ahmedabad-380009, India

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Storm-time increases in the ionospheric total electron content

CHANGES in the total electron content of the mid-latitude ionosphere during the first day of each of 20 geomagnetic storms have been monitored by Papagiannis *et al.*¹. They have shown that during the positive phase of the storms the magnitude of the increase in total electron content exhibits a very pronounced maximum near sunset. Although Rishbeth and Hanson² have shown that plasma convergence in the F region itself cannot account for the observed increases, an inward meridional $\mathbf{E} \times \mathbf{B}$ drift of the plasma may compress the $\mathbf{H}^+$ gas at great heights^{1,2}, giving rise to a field-aligned flow of $\mathbf{H}^+$ into the ionosphere. We have investigated the consequences of field-aligned $\mathbf{H}^+$ flow by integrating the F region–protonosphere equations of motion and continuity for $\mathbf{O}^+$ and $\mathbf{H}^+$ along magnetic tubes that are undergoing $\mathbf{E} \times \mathbf{B}$ drift. The range of integration is from the lower F region to the equatorial crossing point of the magnetic tube, the method of integration being essentially that of Moffett and Murphy³. Our results support the idea that an influx of ionisation from the protonosphere may be partly responsible for some storm-time increases in ionosphere content.

An important feature arising from our investigation is the relationship between the meridional $\mathbf{E} \times \mathbf{B}$ drift of the plasma and west-east $\mathbf{E} \times \mathbf{B}$ drift relative to the Earth. This relation results from the assumption of an incompressible magnetic field. Rishbeth and Hanson² and Murphy⁴ have shown that for a dipole field, at the equatorial crossing point of the field line,

$$\nabla \cdot \mathbf{v}_{em} = -6v_{1eq}/r_{eq} \quad (1)$$

where v_{1eq} is the value of the meridional component of $\mathbf{v}_{em} = \mathbf{E} \times \mathbf{B}/B^2$ at the equatorial crossing point (v_{1eq} being positive in the radially outwards direction) and r_{eq} is the geocentric distance of the equatorial crossing point. The derivation of this expression requires the conditions $\nabla \times \mathbf{E} = 0$ and $\nabla \times \mathbf{B} = 0$. The first of these arises from conservation of the magnetic field. The second is consistent with the dipole field approximation and is justifiable provided electric currents are small enough. Note that equation (1) is correct even if a west-east drift occurs, as will usually be the case.

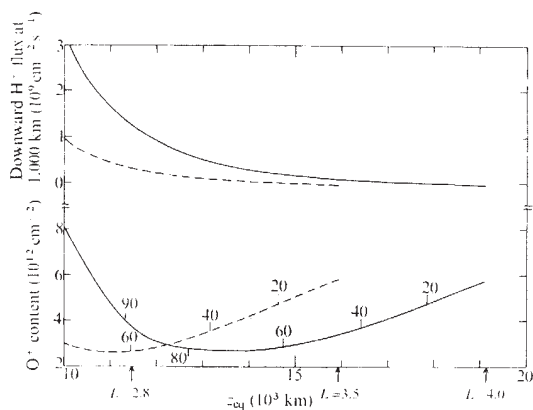


Fig. 1 Plots of the O^+ content in a tube with base area 1 cm^2 at 200 km and of the downward H^+ flux through the 1,000-km level as a function of equatorial crossing height, z_{eq} , of the magnetic tube. —, Results when $L = 4.0$ initially; ---, results when $L = 3.5$ initially. The plasma temperature at the equatorial crossing point is always $\leq 6,000 \text{ K}$; no account is taken of the increase in temperature that will arise from plasma compression. The numbers on the curves give the local time (min) that has elapsed since the tube started drifting.

An alternative expression for $\nabla \cdot \mathbf{v}_{em}$ can be derived by resolving $\mathbf{v}_{em}$ in the meridional and west-east directions:

$$\mathbf{v}_{em} = v_1 \hat{\mathbf{n}} + v_\phi \hat{\boldsymbol{\phi}} \quad (2)$$

where $\hat{\mathbf{n}}$ is a unit vector normal to $\mathbf{B}$ in the meridional plane and $\hat{\boldsymbol{\phi}}$ is a unit vector in the west-east direction, ϕ being the local hour angle and also the azimuthal angle of spherical polar coordinates. At the equatorial crossing point of the field line this alternative expression takes the form^{5,6}

$$\nabla \cdot \mathbf{v}_{em} = \frac{4v_1^{eq}}{r_{eq}} + \frac{\partial v_1^{eq}}{\partial r_{eq}} + \frac{1}{r_{eq}} \frac{\partial v_\phi^{eq}}{\partial \phi} \quad (3)$$

where v_ϕ^{eq} is the value of the west-east plasma drift velocity relative to the Earth at the equatorial crossing point.

Equating equations (3) and (1) gives

$$\frac{\partial v_\phi^{eq}}{\partial \phi} = 2v_1^{eq} - r_{eq} \frac{\partial v_1^{eq}}{\partial r_{eq}} \quad (4)$$

The significance of equation (4) for our purpose is that a uniform, inward meridional drift (giving a negative v_1^{eq}) tends to make v_ϕ^{eq} negative. A negative value for v_ϕ^{eq} means that the plasma is rotating more slowly than the Earth. The time taken by the plasma to drift from a position with a particular hour angle ϕ_1 to a position with a later hour angle ϕ_2 may be much greater than the local time between ϕ_1 and ϕ_2 . If a downward field-aligned flux from the protonosphere is caused by an inward drift, then the effect of this flux on the F region is magnified by the increase of time available for a given observing period of local time.

Equation (4), of course, gives only the rate of change of v_ϕ^{eq} ; the value of v_ϕ^{eq} when the inward drift commences is important. In our calculations we have arbitrarily taken $v_\phi^{eq} = 0$ at the start of the inward drift, since indeed a negative initial value for v_ϕ^{eq} would increase the local-time effects of the downward H^+ flow.

We adopted a time-independent Jacchia model atmosphere, with an exospheric temperature of 1,000 K. The linear loss coefficient for O^+ at 300 km was $3.2 \times 10^{-4} \text{ s}^{-1}$ and a horizontal

equatorward wind in the neutral air with velocity 150 m sec^{-1} was assumed. The concentration of neutral hydrogen at 400 km was taken to be $10^6 \text{ atoms cm}^{-3}$ and the rate coefficient for $H^+ - O$ charge transfer to be $1.4 \times 10^{-9} \text{ cm}^3 \text{ s}^{-1}$ (ref. 7, and E. E. Ferguson, private communication). Typical values of electron and ion temperatures for heights less than 1,000 km were taken from Brace *et al.*⁸ and Evans⁹. We have pointed out (in a paper to be published) that increases in T_e and T_i at great altitudes tend to increase the downwards field-aligned flow of H^+ , and it has been noted by Rishbeth and Hanson² that inward $\mathbf{E} \times \mathbf{B}$ drift will tend to heat the plasma by compression. We have chosen to take, above 1,000 km, T_e equal to T_i and a temperature gradient with height of 2 K km^{-1} until a temperature of 6,000 K is reached. An estimate of the compression heating shows that this is a very conservative value for the plasma temperature at great altitudes. Possible production of O^+ by photoionisation of atomic oxygen was ignored, so that any increases in O^+ content may be ascribed to influx of H^+ .

In our investigation we examined the behaviour of the plasma in two tubes of plasma. One tube (tube 1) underwent $\mathbf{E} \times \mathbf{B}$ drift from $L = 3.5$ to $L = 2.8$, the other (tube 2) from $L = 4.0$ to $L = 2.8$. In both cases the plasma was given a meridional drift velocity of $v_1^{eq} = -1 \text{ km s}^{-1}$. This value is based on the work of Park¹⁰. He has interpreted whistler observations of the draining of H^+ from tubes of plasma during and after a magnetic storm as evidence for inward drift of the tubes. The initial value of O^+ content above 1 cm^2 at 200 km was $5.8 \times 10^{12} \text{ cm}^{-2}$ in both tubes. The initial O^+ contents in the tubes were taken to be identical to isolate $\mathbf{E} \times \mathbf{B}$ compression effects from those arising from spatial gradients in electron concentration in the F region that may initially be present. It should be noted that Rishbeth and Hanson² have suggested that concentration gradients in the F region at initial L values of the tubes may contribute to the storm increases seen at lower L values. Our initial H^+ contents were proportional to the volumes of the tubes.

If the tubes start drifting at local time $t_0 = 0$, tube 1 arrives at $L = 2.8$ (the L value of the observations in ref. 1) at local time $t_1 = 1 \text{ h}$, whereas tube 2 arrives at $L = 2.8$ at local time $t_2 = 1 \text{ h } 30 \text{ min}$. It is found that at $L = 2.8$ the O^+ content in tube 1 is $2.62 \times 10^{12} \text{ cm}^{-2}$ and in tube 2 $3.72 \times 10^{12} \text{ cm}^{-2}$. Thus an observer at $L = 2.8$ will see during local time $t_2 - t_1 \approx 30 \text{ min}$ an increase of $1.1 \times 10^{12} \text{ cm}^{-2}$ in O^+ tube content. Figure 1 shows the behaviour of the plasma in the flux tubes as a function of L value as we follow the tubes.

This result demonstrates at least that, in discussion of magnetic storm increases, ionisation flow from the protonosphere must be taken into account when inward $\mathbf{E} \times \mathbf{B}$ drift occurs.

R. J. MOFFETT
J. A. MURPHY*
G. J. BAILEY

Department of Applied Mathematics and
Computing Science,
University of Sheffield,
Sheffield S10 TN2, UK

Received October 7, 1974.

*Present address: University of Aston in Birmingham, Gosta Green, Birmingham B4 7ET, UK.

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Precambrian geomagnetic field reversal

THE geomagnetic field has reversed many times. Numerous polarity transitions of Cainozoic age, together with very few of Mesozoic and Palaeozoic age, have been studied in detail and the studies indicate that the processes involved have remained relatively unchanged in time. Here we report the first detailed study of a Precambrian reversal.

As part of a palaeomagnetic survey (D.K.B., M.E.E., A. B. Reid, and E. W. McMurtry, unpublished) of Proterozoic sediments in the East Arm Fold Belt of Great Slave Lake, 45 stratigraphically distinct sites have been sampled across a continuous 600-m section. The section consists of steeply dipping, finely bedded, red siltstones in the lower part of the Stark Formation of the Great Slave Supergroup¹. Available K/Ar dates from associated intrusive bodies place the Stark Formation in the age range 1,650–1,800 Myr (ref. 1) though palaeomagnetic data may indicate a slightly younger age. (D.K.B., M.E.E., and E. W. McMurtry, unpublished). Lithologically, the section is very uniform except for many fine green bands which were generally avoided during sampling. Detailed palaeomagnetic studies using thermal and alternating field treatment indicated a marked uniformity in the magnetic properties; all of the specimens are very hard magnetically and possess well defined haematite Curie points.

We have not found any strong evidence that the remanence is primary. It is possible that the red beds could have been remagnetised when they were reheated during the emplacement of the nearby MacKenzie intrusions about 1,200 Myr BP (ref. 2). Our observations do not, however, support that idea. The intrusions were emplaced after the folding of the Great Slave Supergroup and the palaeomagnetic pole which we have obtained is 70° from the MacKenzie pole before bedding corrections are applied. As the MacKenzie intrusions represent the most recent igneous event in the East Arm Fold Belt it seems that the red beds acquired their remanence before 1,200 Myr BP. Other workers³ have reached the same conclusion regarding the possible remagnetisation of other red beds in the East Arm Fold Belt. Furthermore, the presence of a reversal and transition zone such as that described here

Fig. 1 Stratigraphic profile of thermally cleaned, site mean directional and site mean intensity data for the Stark Formation, after correction for geological dip. Each site is represented by 2, 3 or 4 samples. *a*, Remanent intensity (*J*); *b*, inclination below horizontal; *c*, declination east of true north. Error bars indicate the standard error of the mean. Dotted line, five-point running mean of *J* omitting two non-red sites which gave consistent, though weak, results.

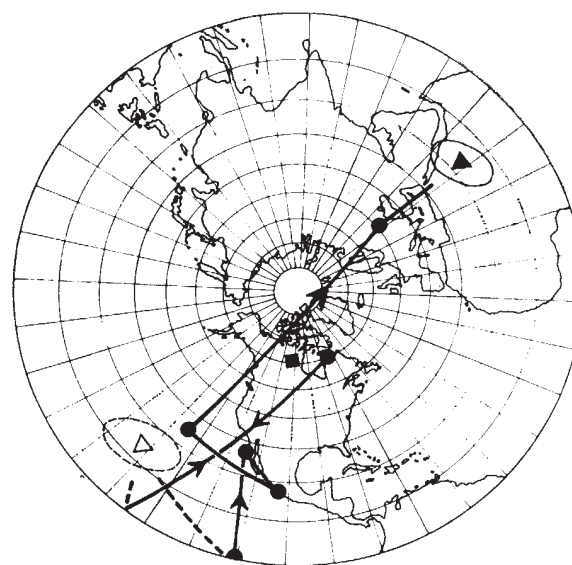
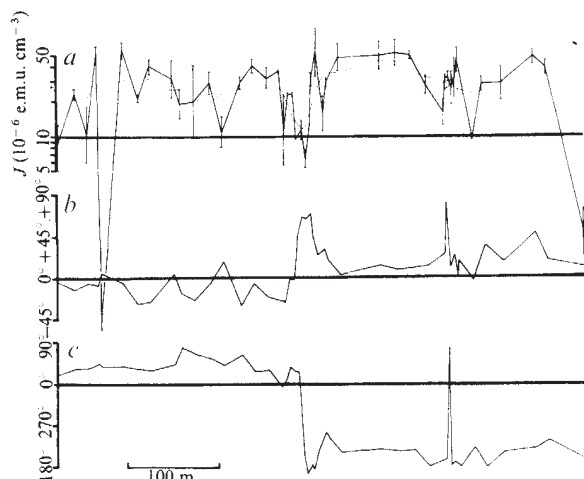


Fig. 2 Virtual geomagnetic poles from the Stark Formation after dip correction. Triangles, stable normal (19 sites) and reversed (20 sites) poles with 95% ovals of confidence; circles, intermediate poles. Square is sampling location. Closed (open) symbols and solid (dashed) lines are on the Northern (Southern) Hemisphere. Wulff net.

indicates that secondary components of magnetisation which post-date the initial magnetisation are not significant⁴. We therefore feel that it is reasonable to assume that the magnetisation is primary.

The most prominent features of the thermally cleaned site mean directions and intensities (Fig. 1) are the polarity reversal which occurs about mid-way through the section and the apparent field excursion in the lower third of the section. We assume that the remanent intensities are log-normally distributed^{5,6}, and we have shown geometrical means and the associated standard errors. This assumption is, however, not critical as the arithmetical means lead to the same conclusions. Figure 2 shows the corresponding virtual geomagnetic poles (VGPs) for the reversal and excursion; only the overall means for the rest of the section are indicated. A number of features of the reversal are immediately apparent: (1) it occurs across approximately 25 m of section (Fig. 1 *b* and *c*); (2) an approximately great circle path is followed by the transitional VGPs (Fig. 2); (3) the pre- and post-transitional VGPs are essentially antipodal (Fig. 2); (4) the remanent intensity decreases by a factor of 2 or 3 within the transition zone (Fig. 1*a*); (5) the reduction in intensity covers approximately the same time interval as the reversal in direction, though there is a suggestion of reduced but perhaps unstable intensity just above and below the transition zone (Fig. 1*a*).

The field excursion occurs across about 5 m of section approximately 150 m below the reversal (Fig. 1). It represents an excursion by the palaeopole of about 80° from the stable direction above and below it, and the VGP of the excursion lies close to the great circle described by the path of the transitional VGPs (Fig. 2). There is a suggestion of reduced and unstable remanent intensity in the vicinity of the excursion but the excursion itself does not show reduced intensity (Fig. 1).

Accepting the appropriate sedimentation rates given by Kukal⁷ the transition covers 10⁴–10⁵ yr, which implies that either the reversal took considerably longer than the 1,000–2,000 yr observed for more recent transitions⁸ or that the sedimentation rate was a factor of 10–100 higher than expected, or some combination of these two alternatives. The excursion occurred across only 5 m of the section, which—if the sedimentation rate was constant, as is suggested by the uniform lithology—

represents less than 1/5 of the time taken for the transition to occur.

The great circle pole path, the antiparallelism of the transition, and the reduced intensity are features common to many but not all of the more recent reversals which have been studied in detail⁹⁻¹⁴. The relationship between the remanent intensity of rocks and the palaeointensity of the geomagnetic field is not necessarily simple. The uniformity of the section both lithologically and magnetically suggests, however, that the coincidence of the reduced remanent intensity with the transition zone is more than accidental. The use of thermally cleaned results in this context is not critical as the uncleaned intensity profile exhibits the same major features.

These observations are relevant to a discussion of the mechanism of geomagnetic field reversal. There are two extreme possibilities: decay and subsequent antiparallel re-establishment of the dipole field, or a 180° dipole 'flip' with no change in strength. The palaeolatitude of the sampling site is 8°, and abundant halite casts offer independent evidence of a tropical climate. At such low latitudes a 'flip' reversal could give rise to a spectrum of intensity profiles ranging from no change to an increase by a factor 2, depending on the meridian followed by the pole. In the present case the transitional pole path passes close to the sampling site and an increase in intensity could therefore be expected. The clear minimum actually observed thus provides strong evidence of a real decrease in the dipole moment during the reversal.

Several workers¹⁵⁻¹⁷ have noted that the period of reduced intensity during recent polarity transitions is longer than the time needed for the direction to reverse, although others have found that these periods coincide¹⁸. It is difficult to place this Precambrian reversal in either class but there is a suggestion of very rapid variations in intensity immediately before and after the change of direction, such as has been seen in more recent reversals¹⁹.

The term 'field excursion' or 'systematic deviation' is usually applied to short duration deviations in pole position, which are greater than 40° or 50° but significantly less than 180° and which are not immediately associated with field reversals²⁰⁻²². The field excursion reported here fits this description but we stress that it is represented by a single site mean. The reduced and/or unstable intensity near the excursion supports findings^{21,23-25} which correlate low latitude poles with reduced intensity.

Perhaps the most significant point about the field excursion is its location near the transition pole path, which suggests that, though it occurred a significant time before the reversal, it is perhaps not wholly unrelated to it. Some coincidence of great circle paths described during several separate reversals⁹ and during distinct field excursions²⁵ has been observed in the recent palaeomagnetic record. The result reported here is consistent with these results and with the hypothesis that field excursions represent aborted reversals²⁰. It also provides support for the existence of long lived non-dipole sources²⁵ or stable equatorial dipoles⁹.

Whatever is the exact mechanism involved in geomagnetic field reversals—dipole decay or dipole flip-over, or a combination of these—the data reported here indicate that the process has apparently remained essentially unchanged for nearly 2×10^9 yr of the Earth's history. The only significant difference involves the possibility that the transition occupied a considerably longer time interval than more recent reversals⁸. These observations place additional limits on the history of the geomagnetic field and, by implication, the thermal evolution of the Earth's core.

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D. K. BINGHAM
M. E. EVANS

*Institute of Earth and Planetary Physics,
University of Alberta,
Edmonton, Alberta, Canada*

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Search for compression before a deep earthquake

DZIEWONSKI and Gilbert¹, using a moment tensor representation of the seismic source, presented evidence for precursive compression before deep earthquakes. For the Colombian earthquake of July 31, 1970 (17:08:05.4 GMT, 1.5° S, 72.6° W, $h > 650$ km), they concluded that a large precursive compression occurred in the focal region, beginning about 80 s before the origin times as determined by ordinary P wave travel times.

These results were, however, obtained from data derived from free oscillations with periods longer than 70 s, and so the time resolution is inevitably limited. Moreover, the phase information contained in the free oscillation data is somewhat obscured by the lateral heterogeneity of the Earth. It is therefore important to search for the existence of precursors by using an independent set of data. Thus, we have examined carefully the direct displacement field caused by a precursor. Specifically, we examined seismograms of the Colombian earthquake of July 31, 1970 and compared these observations with synthetic seismograms of the proposed precursor (Figs 1–4).

The epicentre of the July 31 earthquake is located relatively close to roughly a dozen World Wide Standard Seismograph Network (WWSSN) stations and also close to a station at Naña, Peru. The stations all lie between 6.07° and 16.0° from the epicentre. The wavelength of such a slow precursor is about 1,000 km, and so the Earth can be modelled by a homogeneous medium for our synthetic seismogram computations at these short distances.

For a contraction source with time function $\chi(t)$, the radial displacement in the medium is given² by;

$$u_r = \frac{M}{4\pi\rho\alpha^2} \frac{d}{dr} \left(\frac{1}{r} \chi(t-r/\alpha) \right) \\ = \frac{-M}{4\pi\rho\alpha^2} \frac{1}{r^2} \left[\chi(t-r/\alpha) + \frac{r}{\alpha} \chi'(t-r/\alpha) \right] \quad (1)$$

where α = compressional velocity; ρ = density; r = radial distance; and M = moment of inertia.

The first and second terms in the brackets represent the near-field and far-field displacements, respectively. The time function

of the proposed precursor can be described adequately by the function:

$$\chi(t) = \begin{cases} 0 & t \leq -\tau_1 \\ \frac{1}{\tau_1 + \tau_2} \left[t + \frac{\tau_1}{\pi} \sin \frac{\pi}{\tau_1} (t + \tau_1) \right] & -\tau_1 \leq t \leq 0 \\ \frac{1}{\tau_1 + \tau_2} \left[t + \frac{\tau_1}{\pi} \sin \frac{\pi}{\tau_2} (t - \tau_1) \right] & 0 \leq t \leq \tau_2 \\ 1 & t > \tau_2 \end{cases} \quad (2)$$

where τ_1 is the precursor time constant, τ_2 the time constant of the post-seismic deformation, and $t = 0$ refers to the 'origin time'. This time function avoids discontinuities in displacement, velocity and acceleration at both $t = \tau_1$ and $t = \tau_2$, and represents one of the smoothest time functions for a given time constant. Substituting this time function into equation (1), doubling the result to account for the free surface effect, convolving it with the appropriate instrument functions, and including the necessary cosine factors to account for the azimuth and angle of incidence terms, we obtain the synthetic seismograms. In our present computation we used $M = 5.0 \times 10^{27}$ dyne cm, the value suggested by Dziewonski and Gilbert¹, $\alpha = 8.5 \text{ km s}^{-1}$ and $\rho = 3.5 \text{ g cm}^{-3}$ for the average upper

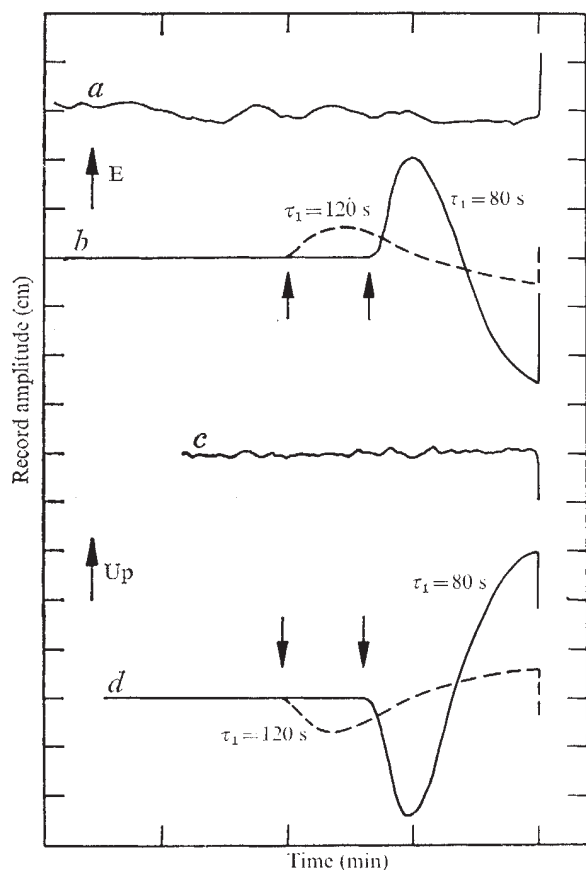


Fig. 1 Long period, WWSSN seismogram from the QUI station (for July 31, 1970). E-W record: *a*, observed trace; *b*, synthetic trace. Vertical record: *c*, observed trace; *d*, synthetic trace. Arrows next to the synthetic traces (*b* and *d*) indicate precursor onsets. All traces end with the impulsive onset of the normal P wave. Peak magnification=3,000; $\Delta=6.07^\circ$.

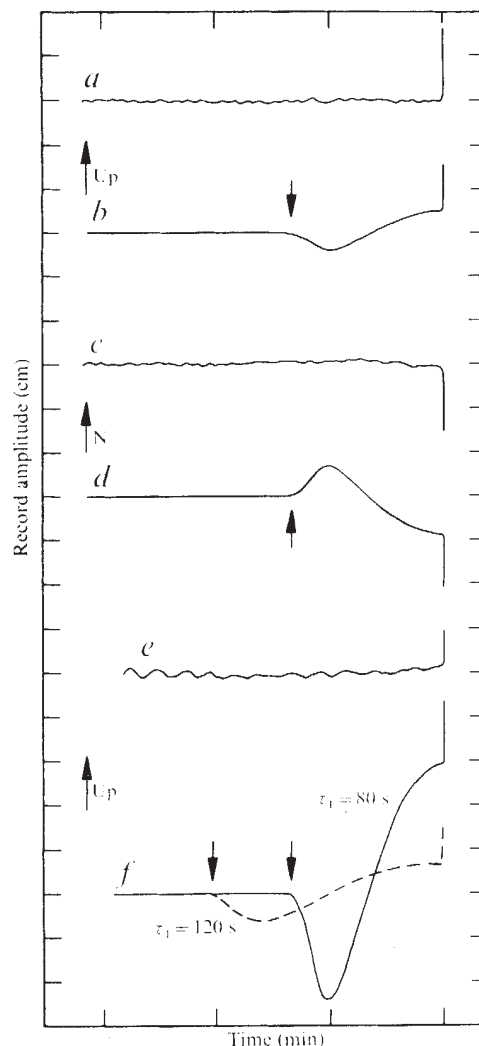


Fig. 2 Long period WWSSN seismograms. *a-d*, ARE records: peak magnification=1,500; $\Delta=14.94^\circ$. Vertical: *a*, observed; *b*, synthetic. N-S: *c*, observed; *d*, synthetic. BOG records: peak magnification=3,000; $\Delta=6.23^\circ$; *e*, observed; *f*, synthetic. Arrows next to the synthetic traces indicates precursor onsets.

mantle. For τ_1 and τ_2 , we consider three cases: (1) $\tau_1 = \tau_2 = 80 \text{ s}$, the value for the precursory time constant suggested in (the abstract of) Dziewonski and Gilbert¹; (2) $\tau_1 = 120 \text{ s}$, and $\tau_2 = 160 \text{ s}$; in this case, $d\chi(t)/dt$ matches the isotropic moment rate tensor suggested by Dziewonski and Gilbert¹ (Fig. 5); and (3) $\tau_1 = \tau_2 = 160 \text{ s}$; this last case represents a more gradual precursor which Dziewonski and Gilbert (personal communication) have suggested might be more appropriate (see Fig. 5).

Long period, vertical seismograms from five WWSSN stations—ARE, BOG, CAR, LPB, and QUI—were available to us. Long period, horizontal records from three of these stations—ARE, LPB, and QUI—were also examined. These seismograms and the corresponding synthetic seismograms are shown in Figs 1, 2, and 3. Recordings of the long period strain network and of the vertical pendulum seismometer at Naña, were also used in our investigations. These records and the respective synthetics are shown in Fig. 4. The very long period information on the strain record indicates tidal strains and some minor surface wave effects from an earlier earthquake in the South Pacific. Neither of these strains was included in the

synthetic strain record. The observed strain record has been fitted with a parabola for the 80 min preceding the onset of the main phase which closely approximates the tidal period. The synthetics for case (1) predict very large precursor amplitudes, as much as 6 cm peak to peak, on a WWSSN instrument operating at a peak magnification of 3,000. We were, however, unable to identify any trace of such an arrival on any of the

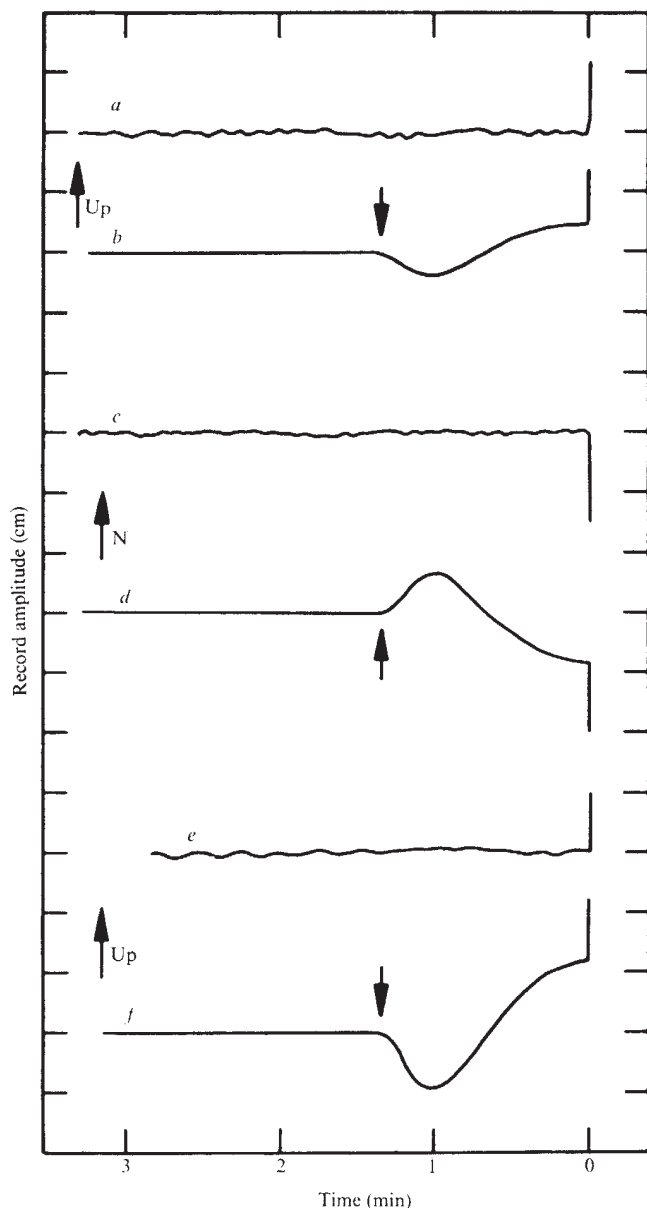


Fig. 3 Long period, WWSSN seismograms. *a-d*, LPB records: peak magnification—1,500; $\Delta=15.61^\circ$. Vertical: *a*, observed; *b*, synthetic. N-S: *c*, observed; *d*, synthetic. CAR records: Peak magnification=3,000; $\Delta=13.15^\circ$; *e*, observed; *f*, synthetic. Arrows next to the synthetic traces indicate precursor onsets.

ten seismograms examined. Thus, if the precursor time constant is 80 s, the moment must be much less than that assumed here, probably less than 5.0×10^{26} dyne cm. For case (2), since the response curve for the Naña strain network is almost flat for $T < 600$ s, the predicted amplitude is reduced only 30% from case (1) (Fig. 4A). In the case of the WWSSN instruments, however, we are dealing with the steep portion of the response

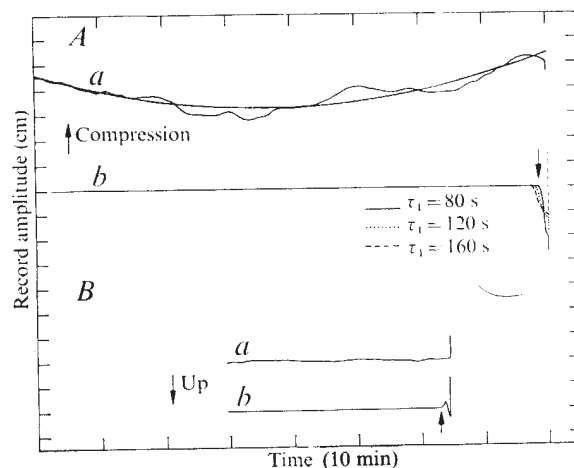


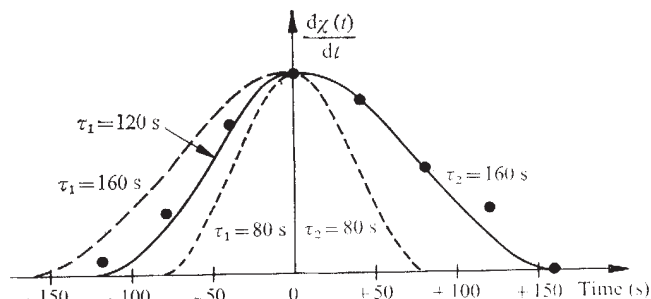
Fig. 4 *A*, Observed (*a*) and synthetic (*b*) strain records for the Caltech north-east strain network at Naña, Peru. Tidal effects removed from the synthetic. Small arrow indicates precursor onset; $\Delta=11.21^\circ$. Note the change of time scale between this figure and the preceding three. *B*, Observed (*a*) and synthetic (*b*) seismograms for Caltech vertical pendulum seismometer at Naña, Peru. Arrow indicates precursor onset; $\Delta=11.21^\circ$. Note that the polarity is reversed.

curve (ω^3) and the amplitude is greatly reduced by the increase in τ_1 from 80 to 120 s. Nevertheless, two nearby stations, QUI ($\Delta = 6.07^\circ$) and BOG ($\Delta = 6.23^\circ$), still show significant amplitudes (Figs 1 and 2). Thus, on the basis of Naña, QUI and BOG, we may conclude that the isotropic moment must be significantly smaller than the suggested value, probably less than 10^{27} dyne cm. For case (3), the precursor signal is almost undetectable at all stations except Naña where the signal is still significant (Fig. 4A). If the precursory time is longer than about 200 s, the proposed isotropic precursor would be hardly detectable. This time constant is somewhat longer than that suggested by Dziewonski and Gilbert¹, but in view of the discrete time interval of about 40 s used by them it might be within the permissible range.

We conclude that if the precursor time constant is less than 120 s, the isotropic moment must be less than 10^{27} dyne cm or, if the precursor moment is 5.0×10^{27} dyne cm, the lower bound of the precursor time is 200 s.

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Fig. 5 ●, Moment rate tensor ($d\chi/dt$) data of Dziewonski and Gilbert¹. The solid and dashed curves indicate the various moment rate tensors used in this study.



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ROBERT S. HART
HIROO KANAMORI

Seismological Laboratory,
California Institute of Technology,
Pasadena, California 91109

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DRS DZIEWONSKI AND GILBERT REPLY—We have stated that for two deep earthquakes the hypocentral region begins to experience compression about 80 s before the origin time determined from the first arrivals of P waves. This statement was based on an estimate of the time at which the isotropic moment rate begins to exceed the noise level visible in the deviatoric part of the moment rate tensor (Fig. 2 of ref. 1). The figure shows that isotropic moment rate is other than zero at times earlier than -80 s, and obviously our estimates of the "beginning" time could not have the meaning that Hart and Kanamori assigned to it.

Our measurements were carried out in the frequency domain. Transformation to the time domain required certain arbitrary, although clearly stated, assumptions because the spectrum was incomplete and contained noise. In these circumstances, if one attempts to introduce an analytical source function, the matching of the parameters should be done in the frequency domain. For the source function proposed by Hart and Kanamori² and the isotropic moment rate spectrum shown in Fig. 1 the best match is for a time constant of 160 s; a time constant of 200 s is also consistent with the data if M_0 is allowed to increase by 50%. In either case the amplitudes of the synthetic seismograms would approach the noise level (the amplitudes would decrease by a factor comparable to that resulting from the increase of a time constant from 80 to 120 s).

It is disappointing that the result of the experiment by Hart and Kanamori is inconclusive, because it seems that with the present instrumentation it may be impossible to

confirm by observations in the time domain the occurrence of precursory phenomena such as those described in our paper¹; the Colombian earthquake was the largest event in its depth range during the past 12 yr. Such confirmation would be very important, as at the present time we must rely on the assumption that the observed spectra should be smoothly interpolated to zero frequency. If this assumption were not valid, then compression might not be precursive. The most important result of our paper¹, which is not subject to this assumption, is that there are volume changes associated with deep earthquakes and that their time function must be significantly different from that of the deviatoric (shear) moment tensor.

In the paper by Hart and Kanamori² we notice a long-period trend in the observed seismograms shown in Fig. 1a and c and Fig. 2c and e that agrees with the sign predicted by the theoretical calculations, that is, is consistent with the hypothesis of precursory compression. The same is true for a deflection on the strainmeter recording that occurred just before the earthquake. (The steeply rising pulse immediately following $t = -\tau_1$ on the theoretical seismograms is determined by the properties of the source function at $t = -\tau_1$. This effect could be substantially reduced if Hart and Kanamori had chosen a function that has a more gradual rise.) Even though none of these examples is significant by itself, the fact that there are several of them is intriguing; particularly as there are no adverse cases.

¹ Dziewonski, A. M., and Gilbert, F., *Nature*, **247**, 185 (1974).

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Chemistry and origin of phlogopite megacrysts in kimberlite

STUDIES of the megacrysts occurring in kimberlite have hitherto neglected the mica megacrysts. We have analysed mica megacrysts from one Lesotho and twelve South African kimberlites. The megacrysts, which vary in size from 4 mm to 3 cm, were taken from both 'basaltic' and micaceous kimberlites, and distinguished from the matrix phlogopites by (i) their greater size; (ii) their dark brown, rather than bronze or light brown, colour; (iii) their rounded shape and corroded margins; (iv) distortion of the {001} planes (in some, but not all, megacrysts); and (v) absence of inclusions of matrix phases such as perovskite and spinel which often occur in the matrix micas.

The average and range of compositions of nine selected megacrysts are shown in Table 1, together with the averages of analyses for primary and secondary micas in peridotite xenoliths in kimberlite (ref. 1 and new data) and analyses of micas from four glimmerite nodules in kimberlite (three from the Bultfontein Mine, one from the Wesselton Mine). All the analyses were made by electron microprobe techniques, of which details will be given elsewhere. Nineteen megacrysts were analysed and checked for homogeneity at five or more points; the nine selected megacrysts were homogeneous, or nearly so, whereas the others showed variations of 5% or more in Fe. Inclusion of the other ten megacrysts does not affect the conclusions.

In Fig. 1, we plot TiO_2 against Cr_2O_3 for all our megacrysts and new data on micas from four peridotites, together with comparative data for ten peridotite micas given by Carswell¹. Carswell had distinguished between primary and secondary micas in peridotites by virtue of lower TiO_2 , Cr_2O_3 , Al_2O_3 , MgO and Na_2O , but higher SiO_2 in the primary micas. Our data for primary micas from peridotite fall very close to a field drawn from Carswell's analyses, whereas those for secondary micas extend towards higher TiO_2 but maintain the high Cr_2O_3 content. The same two oxides are shown for micas from the four glimmerite nodules. The lines AA' and

Fig. 1 Comparison of the observed moment rate spectrum with the spectra predicted by equation (2) of Hart and Kanamori² for several values of the time constant τ ($\tau = \tau_1 = \tau_2$).

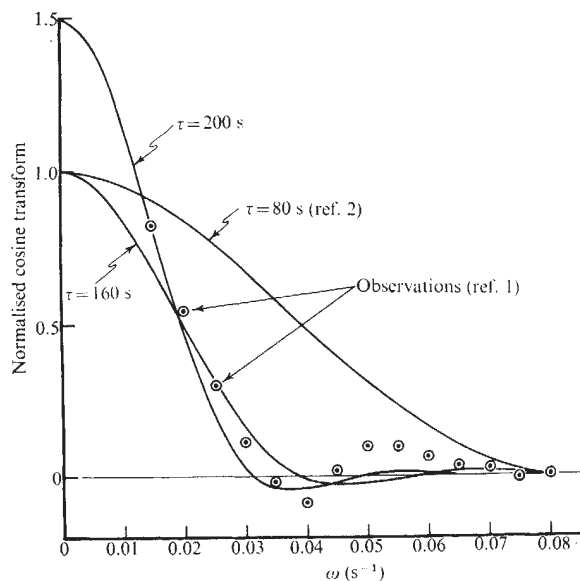


Table 1 Analyses of micas

	1	2	3	4	5	6	7	8	9	
SiO ₂	40.2–42.2	(41.3)	43.4	43.2	44.1	42.4	42.0	38.7	41.3	39.3
TiO ₂	0.6–1.5	(1.06)	1.92	1.09	2.34	1.17	0.53	2.90	0.32	1.11
Al ₂ O ₃	11.2–12.7	(12.1)	9.76	10.5	8.55	10.3	11.6	15.0	12.6	14.9
Cr ₂ O ₃	0.02–0.63	(0.30)	0.25	0.19	0.17	0.31	0.68	1.38	0.70	1.51
FeO	2.9–7.6	(4.9)	7.58	6.08	8.97	6.78	2.56	4.80	2.77	3.06
MnO	0.00–0.03	(0.01)	0.02	0.04	0.03	0.03	0.01	0.02	0.02	0.07
MgO	23.4–25.1	(24.3)	21.7	24.7	20.5	24.3	26.1	22.3	26.8	24.9
NiO	0.03–0.18	(0.11)	0.12	0.23	n.d.	n.d.	0.25	0.12	n.d.	n.d.
CaO	n.d.	n.d.	n.d.	0.00	0.08	0.02	0.00	0.03	0.02	0.03
Na ₂ O	0.05–0.26	(0.16)	0.03	0.14	0.04	0.14	0.18	0.28	0.74	1.22
K ₂ O	10.0–10.6	(10.4)	10.4	9.6	10.5	10.1	10.6	9.10	9.35	8.37
Total	93.3–95.4	(94.6)	(95.2)	(95.7)	(95.2)	(95.5)	(94.5)	(94.7)	(94.4)	(94.4)
mg	0.845–0.939	(0.898)	0.836	0.879	0.803	0.865	0.949	0.893	0.945	0.931
Na/K	0.007–0.038	(0.024)	0.004	0.023	0.006	0.021	0.025	0.046	0.123	0.229

1, Range and mean of nine megacrysts from South African kimberlites (Hololo, Dutoitspan, Wesselton, Star, Monastery, Roberts Victor, Klipfontein, Weltvreden): selected from 19 megacrysts on the basis of chemical homogeneity.

2, 3, 4, 5, Glimmerites from Wesselton (No. 1083-2), Bultfontein (No. 1158, with ilmenite), Bultfontein (No. 1159, with amphibole and clinopyroxene), Bultfontein (No. 1160, with amphibole and rutile).

6, Mean of two primary micas in lherzolite xenolith 1141 from Bultfontein and spinel lherzolite xenolith from De Beers 1197.

7, Mean of two secondary micas in chromite harzburgite (1126, Jagersfontein) and amphibole harzburgite (1368, Monastery).

8, 9, Means of five primary and five secondary micas, respectively, from garnet lherzolite xenoliths in South African kimberlites (from Carswell¹).

BB' at Cr₂O₃ = 0.5 and 1.1 wt % are tentatively chosen to separate the primary and secondary peridotite micas from each other and from the glimmerite micas. For the megacrysts, several features emerge:

(i) None of the megacrysts falls within the range of secondary peridotite micas.

(ii) Four of our megacrysts fall within, or very close to, the field of primary lherzolite micas, and hence might be interpreted as being derived by fragmentation of peridotites containing primary micas.

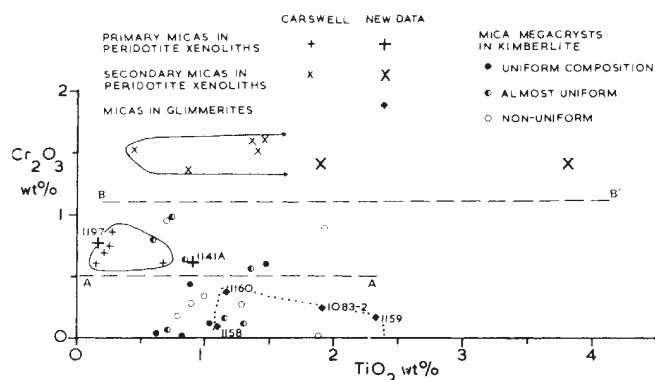


Fig. 1 TiO₂ against Cr₂O₃ for micas. The primary and secondary micas from peridotite xenoliths in South African kimberlites, as selected by Carswell¹, are ringed by solid lines. New data for peridotite xenoliths are keyed by numbers that refer to analyses in Table 1. New data for glimmerite micas are ringed by a broken line and keyed to Table 1. All other specimens are megacrysts in kimberlite of which the average and range are listed in Table 1.

(iii) Three of the megacrysts have similar Cr₂O₃ contents to the primary peridotite micas but higher TiO₂ values; perhaps the field for peridotite micas extends to these higher values of TiO₂, or perhaps TiO₂ was introduced from the host kimberlite.

(iv) Most megacrysts contain less than 0.5% Cr₂O₃, which serves to distinguish these megacrysts from the peridotitic micas and the megacrysts with peridotitic affinities; in addition, the TiO₂ tends to be higher.

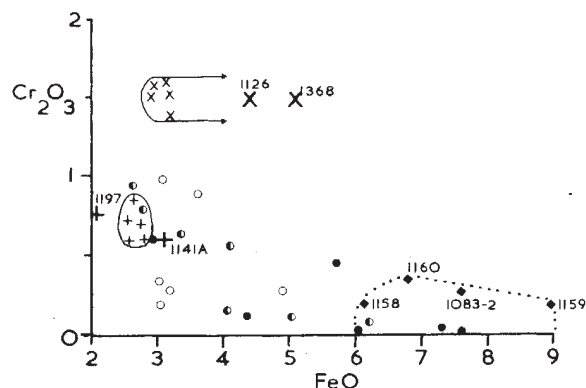
(v) Two of the four glimmerite micas fall directly within the group of 'non-peridotite' megacrysts whereas the other two, although having a higher TiO₂ content than the non-peridotite megacrysts, resemble them in their low Cr₂O₃

values. Here there is the indication of an affinity between the non-peridotite megacrysts and the glimmerites, suggesting that either the megacrysts result from disruption of glimmerite or the glimmerites are accumulations of non-peridotite micas.

A further chemical distinction can be made between the peridotite micas and most of our megacrysts. In Fig. 2, where Cr₂O₃ is plotted against FeO for the same micas, it can be seen that, in addition to the earlier Cr₂O₃ distinction, there are differences in the total FeO contents. Whereas all the primary lherzolite micas (and possible 'peridotitic' megacrysts) contain <3.7% FeO, only three of the megacrysts with low Cr₂O₃ contain <3.7% FeO. The other ten non-peridotite megacrysts have a wide range of higher FeO values from 4.04 to 7.62 wt % FeO. The glimmerite micas also have considerably higher FeO values than the peridotite micas.

In these chemical features (relatively high FeO and TiO₂, and lower Cr₂O₃) that distinguish them from the peridotitic micas, the non-peridotite megacrysts resemble other megacrysts (often referred to as 'discrete nodules') of pyrope garnet, diopside and olivine found in kimberlite; these are distinct from the broadly similar phases in peridotites in the upper mantle because of their large size, their relatively high FeO and TiO₂ content, and their comparatively low amounts of Cr₂O₃ (refs 2–4). In addition our suggestion that the glimmerites may be accumulations of non-peridotite megacrysts and thereby linked in with the overall megacryst suite, is strengthened by the fact that diopside occurring as a minor phase in one of the glimmerites (BD1159, Bultfontein Mine) is a high FeO/low Cr₂O₃ variety that chemically resembles the diopside 'discrete nodules' occurring in kimberlite. The

Fig. 2 FeO against Cr₂O₃ for micas. Ornament as in Fig. 1.



megacryst suite as a whole, in its relatively high FeO and TiO₂, but lower Cr₂O₃, contents seems to be strongly tied in with the kimberlite event when FeO and TiO₂ (together with many other minor and trace elements) were strongly concentrated relative to presumed parental peridotite⁵.

In summary, two distinctive groups of phlogopite megacrysts have been recognised, namely chromium-rich peridotite-derived micas and titanium-rich kimberlite megacrysts. The distinction between the two is important when using the megacrysts for dating purposes as an analysis of a sample consisting of indiscriminated megacrysts would yield a meaningless, hybrid age. In addition, in models for the genesis of kimberlite by fractionation of high pressure phases (the discrete nodule suite) from a primitive ultrabasic parent magma, phlogopite fractionation will have to be taken into account, in addition to that of garnet, pyroxene and olivine.

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J. B. DAWSON

Department of Geology,
University of St Andrews,
St Andrews, Fife, UK

J. V. SMITH

Department of Geophysical Sciences,
University of Chicago,
Illinois 60637

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Flow separation in meander bends

It is generally assumed that downstream flow through meander bends is helicoidal and is accompanied by a transverse, bottom flow component directed towards the inner bank¹⁻³. Thus particles of sediment on a point bar are transported at some angle inwards from the generalised local downstream flow vector^{4,5}. As pointed out by Bagnold³, however, "... a stage

must be reached at which the flow along the inner boundary becomes unstable and breaks away from the boundary, leaving an intervening space occupied by a zone of unstable and confused motion...". The experimental results of Leopold *et al.*⁵ leave no doubt that this phenomenon of flow separation (Fig. 1) can be a highly important feature in river hydraulics. Since 1960, however, no workers have extended these initial experimental ideas into field situations. Existing models of sedimentation and erosion in sinuous channels^{4,5} ignore any possible effects of flow separation. Here we draw attention to examples of flow separation in natural meander bends and attempt to define an empirical criterion for predicting the onset of separation.

Flow separation in meander bends is best expressed as a function of bend tightness and Froude number⁶. Bend tightness is most usefully defined as the dimensionless ratio between meander radius (R_m) and flow width (w) (Fig. 2). This ratio should represent a legitimate scaling factor for both small and large channels since both width and radius bear approximately linear relationships to channel magnitude expressed as meander wavelength⁷. Use of the Froude number (dimensionless) as the other variable allows results from a wide range of channels to be compared.

Our results for the occurrence of flow separation in natural meanders were obtained from intertidal meandering channels in the Solway Firth, Scotland⁸. These are cut into interlaminated muds and silts, and are suspended-load channels according to the terminology of Schumm⁹. Four meander bends were studied, observations and measurements (23 in all) being made during several ebb tides. A wide range of R_m/w : Fr values were obtained (see Fig. 2). The results indicate that flow separation is possible in a range of meanders at quite modest Froude numbers (0.27–0.42). Reaches showing flow separation are well separated from those which do not. The discriminant curve drawn indicates that flow separation is a steeply decreasing function of Froude number as the meander bend becomes less tight. The discriminant curve has been extrapolated to the left in Fig. 2 to take in the experimental and field data of Rozovskii³, where no flow separation was noted, and the field data of Taylor *et al.*¹⁰, where flow separation was observed. In the latter case the Froude number for the flow was quoted only as less than 0.1. Figure 3 shows a replot of experimental

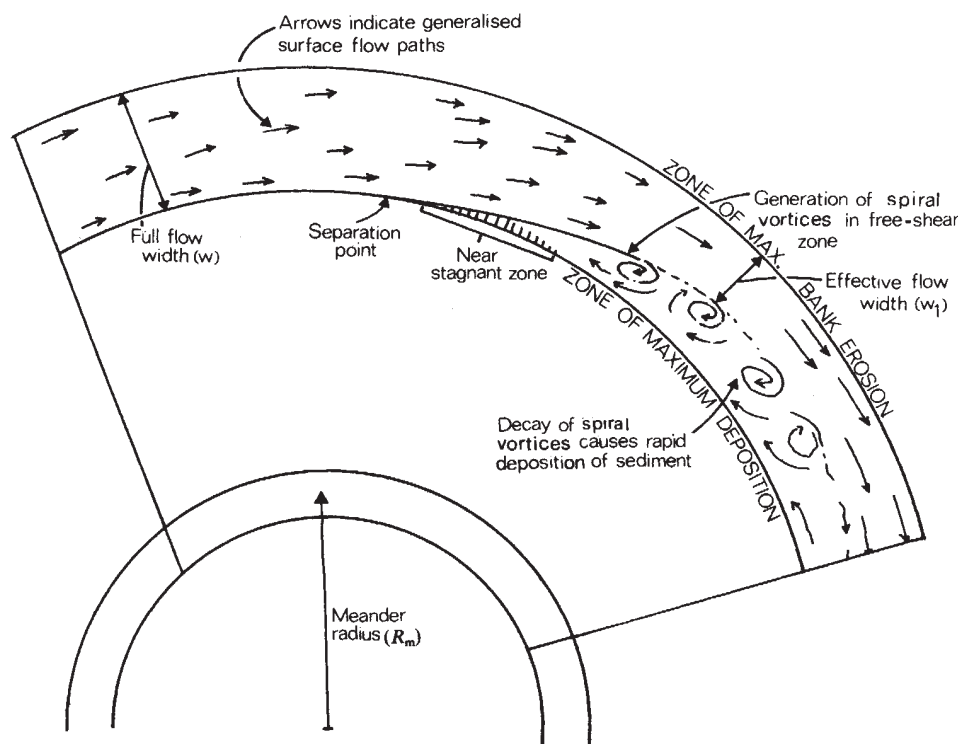


Fig. 1 Sketch to show the main characteristics of separated flows in meander bends.

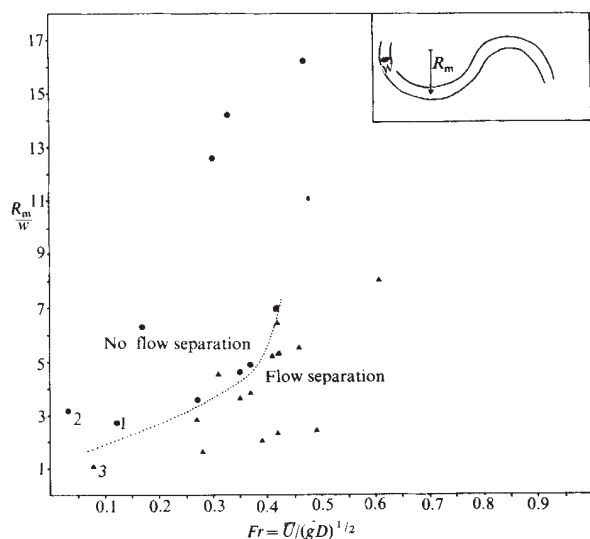
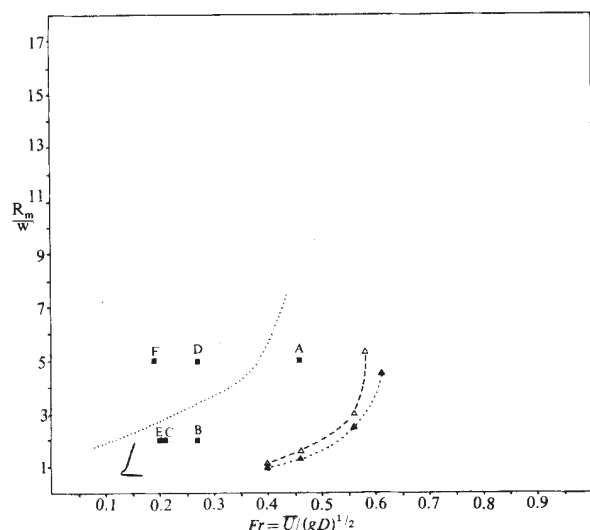


Fig. 2 Dimensionless graph to show the occurrence of flow separation in natural channels. Data points 1 and 2 refer respectively to experimental and field results (R. Desna) showing no flow separation (ref. 3). Data point 3 refers to the River Barwon (ref. 10) where flow separation was observed but where the Froude number is given only as less than 0.1. ●, No flow separation; ▲, flow separation; R_m , meander radius; w , flow width; U , mean velocity; D , flow depth.

data for the onset of spill resistance⁶ as the flow in meander bends becomes locally critical. This plots sensibly to the right of the discriminant curve of Fig. 2 and follows the same general trend. Comparison of field and experimental data confirms that flow separation in meanders is not confined to flows which exceed the spill resistance threshold.

The effects of flow separation in our study reaches were: (1) a decrease in the effective width downstream and opposite the separation point of up to 50%, thus greatly increasing the local velocity and hence the erosion rate for the outer bank (Fig. 1); (2) rapid deposition of suspended silt in the inner

Fig. 3 Dimensionless graph showing the discriminant curve of Fig. 2 (---; depth range = 50–350 mm, Solway Firth) together with curves marking the onset of spill resistance in experimental channels⁶. ▲, Data of ref. 6 for onset of spill resistance in experimental meandering channels (flow depth = 27 mm); ▲, same for channels 20 mm deep. Note the depth effect in the latter curves. Also shown are Allen's model channels⁴ used in his theoretical study of deposition in meander bends (■ (A–F)). We predict that channels A, B, C and E should show flow separation at bankfull discharges.



separation zone (Fig. 1) and development of upstream-facing current ripple bedforms. The boundary between the main downstream channel flow and the more sluggish fluid in the separation zone periodically deforms into spiral vortices. These decay as the separation zone merges back into the general downstream flow at the meander inflection point. The decay and inward movement of eddies associated with these vortices is the cause of the high deposition rates of suspended sediments noted above (up to 20 mm in one ebb/flood cycle).

Our results suggest that flow separation is to be expected in many natural rivers. Of particular interest is the role of the separation eddy in local upstream sediment transport and bedform migration. Such features have been but rarely noted. Their development in the Rivers Mississippi¹¹ and Barwon¹⁰ suggests that flow separation is not restricted to small channels and that the plot in Fig. 2 may be sufficiently valid to warrant general application. Further measurements in natural rivers are needed to confirm and extend the present work.

A further consequence of flow separation is that theoretical models of deposition on point bars that assume a bar-hugging downstream flow cannot be valid for all natural channels. Allen's model channels⁴ are plotted in Fig. 3 and comparisons with our results suggest that several of these should show flow separation during bankfull discharges. This effect might give rise to suites of bedforms and gradations in grain size not predicted by the theoretical analysis.

M. R. LEEDER

Department of Earth Sciences,
University of Leeds,
Leeds, Yorkshire

P. H. BRIDGES

Department of Biological and Earth Sciences,
Derby College of Art and Technology,
Kedleston Road,
Derby

Received October 18; revised December 5, 1974.

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Experimental observation of Abraham force in a dielectric

MINKOWSKI's momentum-energy tensor¹ for material media was severely criticised by Einstein and Laub². The density force on matter according to these authors is:

$$\mathbf{f}_M = \rho \mathbf{E} + \rho \mathbf{v} \times \mu_0 \mathbf{H} + (\mathbf{P} \cdot \nabla) \mathbf{E} + (\mathbf{M} \cdot \nabla) \mathbf{H} - \frac{1}{2} \nabla (\mathbf{E} \cdot \mathbf{P} + \mathbf{H} \cdot \mathbf{M}) + \mu_0 \frac{\partial \mathbf{P}}{\partial t} \times \mathbf{H} + \epsilon_0 \mathbf{E} \times \frac{\partial \mathbf{M}}{\partial t} - \frac{\partial}{\partial t} (\mathbf{D} \times \mathbf{B} - (1/c^2) \mathbf{E} \times \mathbf{H}) \quad (1)$$

$$\mathbf{f}_{E-L} = \rho \mathbf{E} + \rho \mathbf{v} \times \mu_0 \mathbf{H} + (\mathbf{P} \cdot \nabla) \mathbf{E} + (\mathbf{M} \cdot \nabla) \mathbf{H} + \mu_0 \frac{\partial \mathbf{P}}{\partial t} \times \mathbf{H} + \epsilon_0 \mathbf{E} \times \frac{\partial \mathbf{M}}{\partial t} \quad (2)$$

with $\mathbf{D} = \epsilon_0 \mathbf{E} + \mathbf{P}$, $\mathbf{B} = \mu_0 \mathbf{H} + \mathbf{M}$ and using SI units.

Abraham³ assumes a different density force which agrees with equation (2) in the terms depending on time derivatives.

For an isotropic and homogeneous material the relationship between Abraham and Minkowski density forces is¹:

$$\mathbf{f}_A = \mathbf{f}_M + [(\epsilon_r \mu_r - 1)/c^2] \partial(\mathbf{E} \times \mathbf{H})/\partial t$$

the last term (the 'Abraham term') has been the subject of a long and difficult theoretical controversy. Although some experiments, such as those described in refs 5 and 6, seemed at first glance to favour Minkowski's point of view, they could equally well be explained by Abraham's tensor⁷.

A direct experiment has been suggested by Marx and Gyorgyi⁴ to verify the reality of the Abraham term. This has never been carried out, to our knowledge, because of the smallness of the expected effect. Pauli⁸, Møller⁹ and others seemed to be pessimistic regarding the possibility of deciding this annoying theoretical discrepancy by a direct experimental check. But our experiment, a refined version of the one suggested by Marx and Gyorgyi, has conclusively proved that the Abraham term not only exists but is measurable with a reasonable degree of accuracy. This finally resolves the old question in favour of Einstein-Laub and Abraham.

The principle of the experiment was as follows: a disk of barium titanate, of dielectric constant near to 4,000, had a small central hole and the edges coated with aluminium to act as a cylindrical condenser. A long tungsten fibre, 0.2 mm in diameter, held the ceramic disk as a torsional pendulum between the two poles of an electromagnet having a vertical uniform magnetic field of about 10 kgauss in the 3.5-cm air gap in which the disk was suspended. The outer edge of the disk was grounded to the bottom pole of the magnet with a vertical very thin strip of gold which did not sensibly alter the mechanical behaviour of the torsional pendulum nor add any measurable Lorentz force. A focused laser provided a 10-m optical arm to amplify the damped oscillations of the torsional pendulum.

In order to synchronise the applied voltage to the torsional oscillations a signal was obtained from a photo-resistor activated each time the light beam crossed the scale. A sinusoidal voltage was generated with peak value 150 V. This voltage was then applied across the dielectric and could be set in phase or in antiphase with the torsional oscillations.

The mechanical force acting on the disk, according to Abraham, would be given by $(\partial \mathbf{P}/\partial t) \times \mu_0 \mathbf{H}$ where $\mathbf{P}$ is the polarisation of the dielectric. In our case the corresponding torque amplitude was about 2.5×10^{-10} N m. According to Minkowski there would be no torque.

The first problem to be overcome in carrying out the experiment was the reduction of noise, occasioned by vibration in the laboratory, to an acceptable level. This was accomplished by mounting the entire apparatus on a platform weighing about 1 ton which was suspended from the roof by a cable which included a damping element in the form of an automobile tyre. The light beam was broken into four paths by mirrors and the final scale was mounted near the apparatus on the same platform. The periodicity of the support system was about 20 s whereas it was only 2.9 s for the torsional pendulum, so there was no noticeable coupling. The most annoying difficulty was that electrostatic forces appeared between static charges forming on the conductors feeding the condenser and their images in the poles of the electromagnet. These were reduced to an acceptable level by allowing an adequate gap between the ceramic and the poles and reducing the alternating applied voltage. With 300 V (peak-to-peak) the total system noise (electrical plus mechanical) gave a deflection of the light spot on the scale of about 1 cm.

The expected stationary amplitude attributable to the Abraham term is about 3.7 cm. In fact, after 1 h of transient, we measured a steady amplitude of (4 ± 0.5) cm. The effect has been checked by reversing the magnetic field and, in other cases, by changing from phase to antiphase the application of the alternating voltage. The observed changes in the transient oscillation agreed, within the error limits, with the predicted values of Abraham's density force.

In order to obtain a more accurate measurement of the Abraham term we are refining the experiment, adding a vacuum system which should reduce damping by about a factor of 4.

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G. B. WALKER
D. G. LAHOZ

Electrical Engineering Department,
University of Alberta,
Edmonton, Canada

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Thermal effects in the necking of thermoplastics

THE new technique of thermovision has been used to study deformation and fracture processes in polymers^{1,2}. This technique provides a thermal image of the polymer surface, taken by means of natural infrared radiation in the wavelength range 2 to 6 μm . The method also allows an estimate to be made of the surface temperature of a polymer, although the value obtained may sometimes be low because of transparency in the polymer films.

Following our first experiments¹, we recently made a film to demonstrate the formation of hot areas during polymer deformation processes. During this work we observed an apparently novel feature of the normal necking process, using rigid poly(vinyl chloride) and polycarbonate plastics. At a moderately high strain rate (10.0 h^{-1}) we found that with PVC only one end of the necked region of the polymer was heated, that is, all the 'cold' drawing was taking place at one point (Fig. 1), whereas with polycarbonate drawing was distributed between the two ends of the necked region (Fig. 2).

Fig. 1 Thermovision photograph of the necking of PVC. Estimated minimum temperature rise was 9°C .

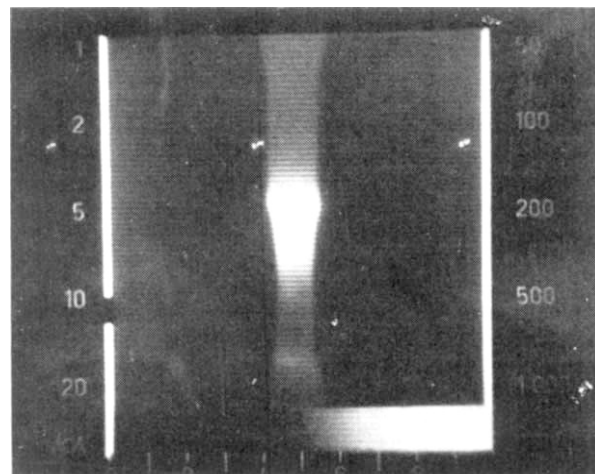
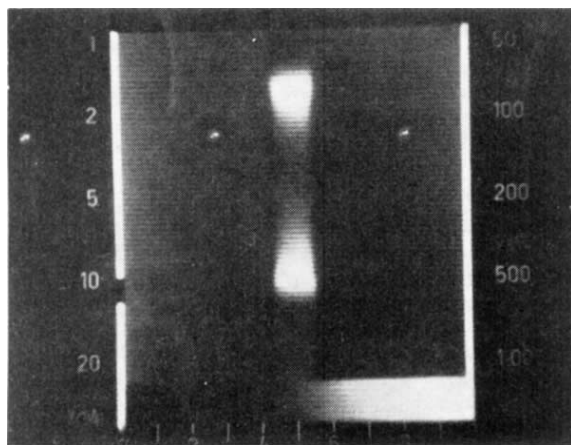


Table 1 Temperature and strain rate sensitivity of the drawing stress σ_D of PVC and polycarbonate plastics

	PVC	Polycarbonate
$\frac{d\sigma_D}{dT}$	(Temperature sensitivity) $-0.71 \text{ MN m}^{-2} \text{ per } ^\circ\text{C}$	$-0.25 \text{ MN m}^{-2} \text{ per } ^\circ\text{C}$
$\frac{d\sigma_D}{d(\ln \dot{\epsilon})}$	(Strain rate sensitivity) 1.5 MN m^{-2}	1.0 MN m^{-2}
Glass transition temperature	67°C	160°C

We suggest the following explanation of this difference in behaviour. With PVC the fall in stress caused by an increase in temperature at the neck greatly exceeds that which would be achieved by the halving of the strain rate which occurs when yielding takes place at each end of the necked region. Thus the reduction in heat losses because of the higher strain rate when

**Fig. 2** Thermovision photograph of the necking of polycarbonate. Estimated minimum temperature rise was 6°C .

straining occurs only at one end dominates the strain rate effect and the plastic strain process becomes localised at one end of the test piece. This explanation is supported by the data given in Table 1; the temperature sensitivity of the drawing stress for PVC is greater than for polycarbonate, whereas the strain rate sensitivity (at low strain rates) is not very different for the two polymers. The increased temperature sensitivity of PVC is, of course, related to its lower glass transition temperature as shown in Table 1.

A. CROSS
M. HALL
R. N. HAWARD

Centre for Materials Science,
University of Birmingham,
Birmingham B15 2TT, UK

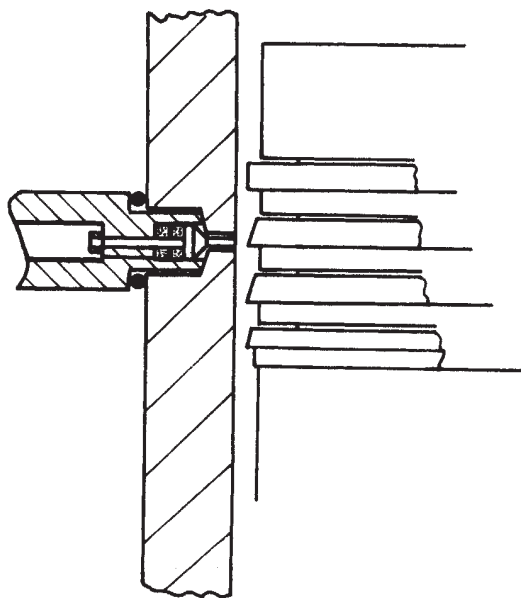
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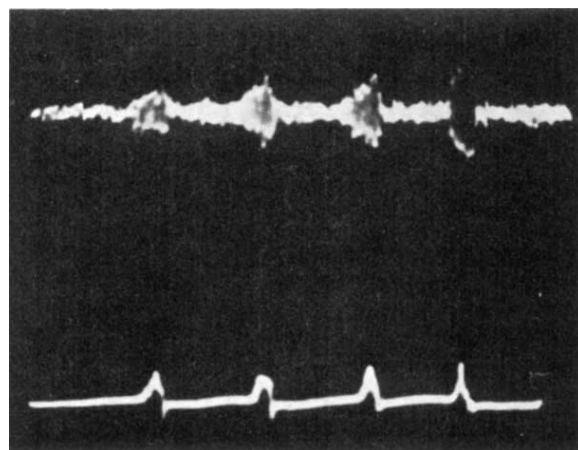
Hydrodynamic pressure under a piston ring

CASTLEMAN postulated¹ that the lubrication of piston rings might be hydrodynamic. Recently it has become possible to measure the oil film thickness directly, using a capacitance transducer². Oil film thicknesses of the order of $1 \mu\text{m}$ have been measured in a small diesel engine; theory predicts thicknesses of

**Fig. 1** The pressure gauge in the liner wall.

the order of $10 \mu\text{m}$. To investigate this discrepancy it was decided to measure the pressure in the oil film, since this provides an independent check on the theory.

A miniature pressure transducer was designed capable of operating in the conditions of a working engine. Essentially the transducer consisted of a cone-shaped plunger which transmitted the pressure from the cylinder face to a piezoelectric crystal (Fig. 1). The plunger was sealed at the front face of the cylinder by a flexible cement, the effective resolving power of the gauge being 0.025 cm . By mounting a capacitance transducer alongside the pressure gauge it was possible to obtain a synchronised oscilloscope display of pressure and film thickness.

**Fig. 2** Output from capacitance and pressure gauge on exhaust stroke.

Experiments have been carried out on a Petter AV1 diesel engine made available by Mr R. P. Langston of the Admiralty Oil Laboratory, Cobham. Figure 2 shows a result taken at 950 r.p.m. on the exhaust stroke. The upper trace is from the capacitance transducer; the presence of the rings is indicated by the out-of-balance signal. The lower trace is from the pressure gauge, the signal level being directly proportional to pressure. The first ring seen, on the left-hand side of the photograph, is the nominally parallel faced compression ring (running-in has

modified the profile to a slight taper), this is followed by two downward scraping taper-faced rings and finally a stepped-scraper ring. These traces show that the pressure distribution does not extend over the whole of the converging gap between the piston ring and the cylinder liner. This is contrary to the assumption of the elementary theory. The rings can be said to be operating in conditions of starved lubrication, presumably caused by the working of the ring-pack.

Details of the method of calibration together with a full account of the effect of engine conditions will be given elsewhere. Although the results do not conform completely with the details of simple theory, it is believed to be the first time that the pressures postulated by Castleman¹ have been observed directly.

S. R. BROWN
G. M. HAMILTON
S. L. MOORE

*Department of Applied Physical Sciences,
The University, Reading, UK*

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Field-desorption microscopy and the atom probe

WE have found unexpected and finely detailed field-desorption patterns, which demonstrate that the yields of metal ions from the surfaces of specimens in the field-ion atom probe are a function of the crystallography and sometimes also of the materials.

The field-ion atom probe¹, in which a field-ion microscope is combined with a time-of-flight mass spectrometer, has been shown to be a useful instrument for the chemical analysis on an atomic scale of metals and especially of alloys²⁻⁴. In an analysis, the field-ion specimen is manipulated to place an image feature over the entrance aperture of the mass spectrometer, which typically subtends only a few image points; a high voltage pulse is then applied to the specimen to desorb

a small amount of material. Ions desorbed from the selected feature pass through the aperture and their mass-to-charge ratios may be deduced from their flight times to a detector. The atomic resolution of the field-ion microscope in principle allows the feature to be chemically analysed atom by atom.

In practice, however, the ion yield is found to be a sensitive function of the position of the selected feature on the specimen surface. Brenner and McKinney⁵ have described systematic 'aiming errors' due to displacements of the trajectories of the field-evaporated metal ions from those of the ions of the image gas.

We have used a simple field-desorption microscope⁶ to investigate these phenomena directly. In this instrument an image is formed of the arrival positions of field-evaporated metal ions at the screen of a conventional field-ion microscope fitted with a micro-channel-plate image intensifier⁷. The image may be recorded directly using large aperture optics and a fast film (Kodak Tri-X or 2475 recording film), with up to 1,550 V applied to the channel plate; under these conditions the detection efficiency for single ions was estimated to be 20 to 50%. Alternatively, the channel plate gain and the lens apertures could be reduced to allow the image to be formed by the evaporation of many layers of the surface without saturation of the film. Proximity focused channel-plate intensifiers were found to be more suitable at very fast evaporation rates than magnetically focused intensifiers, which suffer from space-charge defocusing at the very high currents involved; this is likely also to apply to external image intensifiers.

When fractions of a monolayer of a tungsten surface were pulse evaporated to form the desorption image, micrographs similar to those presented by Walko⁸ and Panitz^{8,9} were obtained. Direct comparison of the field-ion and field-desorption images from the same surface demonstrates the general validity of Brenner and McKinney's conclusions concerning the directions of aiming errors, and allows their accurate measurement for different parts of the specimen surface.

When many planes of tungsten or other metal surfaces were evaporated to form the image, at reduced intensifier gain, very striking and unexpected intensity distributions were

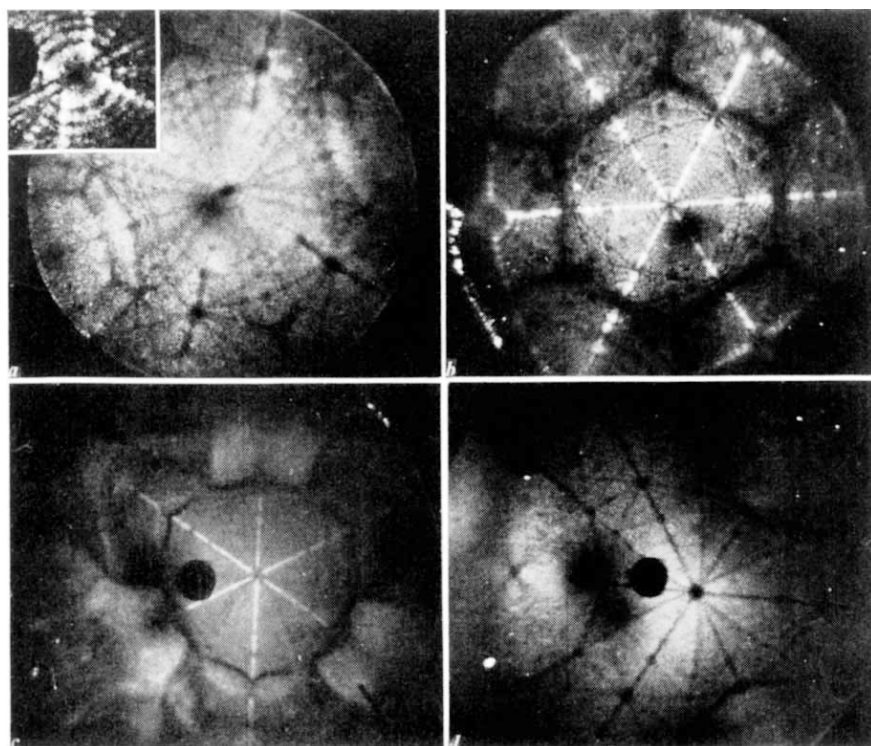


Fig. 1 Field-desorption micrographs at 78 K. *a*, Tungsten, $\langle 110 \rangle$ centred, evaporated by multiple 15-ns pulses; inset continuous evaporation, showing ring structure, zone decoration and radial bright lines towards the $\{211\}$ planes. *b*, Rhodium, a typical f.c.c. pattern. *c*, Nickel. *d*, Aluminium in ultra-high vacuum. The f.c.c. patterns are all continuous evaporations and $\langle 111 \rangle$ centred.

obtained (Fig. 1 a-d). These became more well defined as more of the surface was evaporated, and were reproducible. Only minor variations were introduced by using evaporation pulses of 15 ns, 80 μ s or 500 μ s, or rapid continuous evaporation, or by superimposing many 15-ns pulses. As these patterns have been observed reproducibly under extremely diverse conditions using both proximity and magnetically focused image intensifiers, the possibility of instrumental artefacts (such as channel plate saturation¹¹) may be discounted. The patterns for the more refractory metals (such as W, Ir, Re, Mo) were largely independent of vacuum conditions in the range 2×10^{-10} torr to 5×10^{-8} torr at 78 K. In contrast, the pattern from the non-refractory aluminium was severely modified by as little as 5×10^{-9} torr of hydrogen.

The desorption patterns from b.c.c. metals (such as W, Ta, Mo, Fe) are as varied in character as the corresponding field-ion patterns. Those of the f.c.c. metals (Ir, Au, Rh, Pd), with the notable exception of aluminium, are all very similar, showing bright and dark line contrast with a faint background pattern of dark lines. A similar desorption pattern has been reported recently by Muller and Tsong¹⁰. Nickel is similar in the central region of a (111)-centred specimen, but shows a more complex pattern towards the edges, perhaps due to local mechanical deformation. Aluminium alone shows only dark lines in the desorption image. Dark centres to major planes are commonly seen, as are concentric ring structures centred on such planes (for example, {110} tungsten, {111} for Rh and Al); these structures persist even when hundreds of planes are evaporated to form the image.

We believe that the origins of these desorption patterns lie in short-range migration of atoms¹², induced by polarisation forces in the high electric field, along specific surface directions during the evaporation process; we do not think that differences in local magnification associated with differences in local curvature can alone lead to the observed detailed contrast, and in particular to the bright-line contrast, as originally suggested by Muller for platinum¹⁰. Coulomb repulsion between simultaneously evaporating ions is likely to contribute to the dark centres of major planes, which are more pronounced at high evaporation rates. Whatever the full explanation, we conclude that field-desorption microscopy will lead to a better understanding of the field-evaporation process and is an essential complement to atom-probe field-ion microscopy. This is particularly so for the analysis of very small precipitates and for analysis of segregants at grain boundaries, where we have observed both bright and dark contrast in the desorption micrograph. Provided that such effects are recognised, the atom probe remains the only instrument capable of accurate chemical analysis on an ultra-fine scale.

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A. R. WAUGH
E. D. BOYES
M. J. SOUTON

Department of Metallurgy and Materials Science,
University of Cambridge, Pembroke Street,
Cambridge CB2 3QZ, UK

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Lead in urban street dust

LEAD pollution and its effects on health are matters of general concern^{1,2}. The potential danger to children is now receiving particular attention. Lead in various inorganic forms may be inhaled or ingested, and various sources of ingested lead (for example food, tapwater, paint) are recognised, and tolerable limits have been established. Recently, it was claimed² that for children in urban surroundings the dust of streets and playgrounds is a potentially significant source of lead, and that the lead in such dust can be over 1,000 p.p.m. (0.1%). This is a source of lead which is not immediately controllable, but we consider that its importance as a component of the lead intake of urban children has not been sufficiently assessed.

This investigation has two simple objectives: to determine the lead content of the dust and dirt likely to be encountered by children as a normal part of their environment in Greater Manchester, and to assess the importance of the dust as a component of the lead intake of urban children. Our results show an average lead concentration of 970 p.p.m., with little significant variation either with type of locality (main road, side street, playground, and so on), or with position within the urban area. Samples from nearby rural areas had average concentrations of 85 p.p.m. By comparison, typical values¹ for rural soils in Britain fall in the range 50 to 100 p.p.m., whereas the lead content of 'uncontaminated' soils is ~ 10 p.p.m. Our results for urban dust are similar to those obtained in other cities, for the few studies of which we are aware. In Birmingham, most values (R. Stephens, personal communication) were between 1,000 and 2,500 p.p.m. (average $\sim 1,700$ p.p.m.), the average for Rio de Janeiro³ was 700 p.p.m., and the averages for 77 cities in the USA (ref. 4) were between 1,500 and 2,400 p.p.m.

The 350 urban samples collected were of street dust, surface dirt or soil, from pavements, roadside gutters, children's playgrounds in parks, primary school playgrounds, gardens and waste land, over an area within ~ 10 km of the centre of Manchester, during the period December 1972 to March 1973. (In the south-east, the area sampled extended 15 km.) Twenty-five similar samples were also collected from a children's playground and a park in a rural area ~ 1 km outside the built-up zone, to the south-east of the area and ~ 15 km from the centre of Manchester. The samples were dried at 120° C and homogenised by grinding. Portions (~ 1 g) were boiled with 2 M nitric acid for 30 min. The lead content was determined by atomic absorption spectrophotometry and, for a randomly selected 10%

Fig. 1 Numerical distribution of lead concentration. Numbers above full arrows are percentages of samples below lead concentrations indicated.

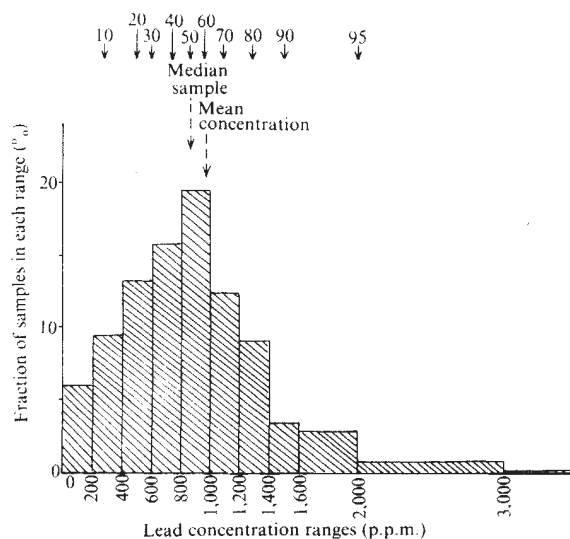


Table 1 Data subdivided according to type of locality (A–D, urban; E, rural)

Type	Definition	No. of samples	Mean Pb concentration (p.p.m.)	Standard deviation
A	Major roads/streets; moderate/heavy traffic 0800–2000	180	1,001	40
B	Minor roads/streets; light/moderate traffic 0800–2000	68	888	57
C	Streets with very light traffic only, and 'play streets'	53	933	186
D	School playgrounds; childrens' play areas in parks, gardens, waste land	49	1,014	206
E	Rural locations; childrens' playground and a 'country park'	25	85	40
A–D	All urban areas	350	970	46

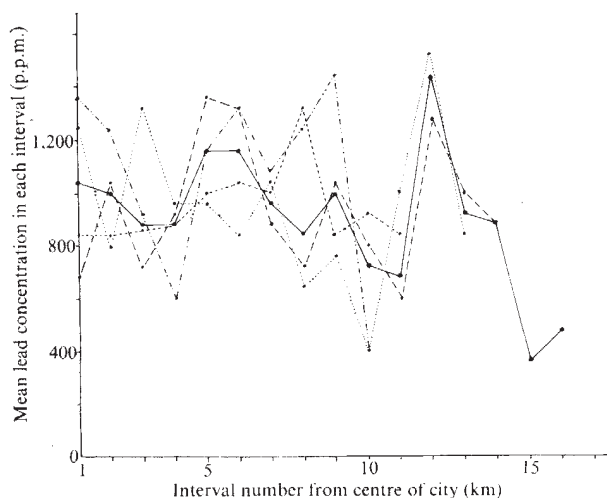
of the samples, by polarography. Agreement between the two methods was always better than $\pm 3\%$.

The average lead concentration in the urban samples was 970 p.p.m. (standard deviation 46); the numerical distribution is shown in Fig. 1. The highest value encountered was 10,200 p.p.m. and the lowest 90 p.p.m. But the higher values were unusual; only 10% of the samples had lead concentrations above 1,500 p.p.m. and only 5% above 2,000 p.p.m. The median value was 860 p.p.m. and the middle 50% of samples ranged from 550 to 1,200 p.p.m.

The urban samples were subdivided into four divisions, according to the type of locality (A–D, Table 1). There was no significant difference between the average lead concentrations in any of these subdivisions. The average lead concentration in the samples from the rural areas was 85 p.p.m., the type of locality being comparable with subdivision D.

We examined the geographical dependence of our analytical data in three ways. First, we subdivided the city into quadrants, and calculated the average lead concentrations for each quadrant. There was no significant difference between quadrants (Table 2).

In the second method we examined the data for a possible relationship between lead concentration and distance from the centre of the urban area. The distance of each sampling point from the centre (St Peter's Square) was calculated and the distances subdivided into 1-km intervals. The average lead concentration for the samples in each interval was calculated,

Fig. 2 Radial distribution of lead concentrations from the centre of Manchester. Quadrants NE, - - - -; SE, ---; SW,; NW, — · — ·; all, ———.

for each quadrant separately and for all samples. The resultant radial distributions (Fig. 2) fluctuated between about 600 and 1,400 p.p.m., with no significant general rise or fall with distance except for a sharp reduction at the distance corresponding to the edge of the urban area.

Third, we attempted to produce a density map of the area. For each 1-km square (National Grid), we compared the average value of the lead concentrations with the map of Greater Manchester. No general pattern emerged, and there was no apparent correlation with major roads or local centres of commercial and/or industrial activity.

It seems reasonable to assume that the bulk of the lead in the urban dust was at some time airborne. The principal sources of airborne lead¹ are probably vehicle emissions (> 90%), smoke from coal and refuse burning, and emissions from various metallurgical and industrial processes. The geographical distribution of lead contamination of the dust seems to support the view that much of the lead is airborne for periods of up to several weeks, and presumably more or less complete mixing of lead from the various sources must occur. As a result, a reasonably uniform dispersal takes place.

Table 2 Data subdivided according to quadrant of city (urban samples only)

Quadrant	No. of samples	Mean Pb concentration (p.p.m.)	Standard deviation
North-east (NE)	43	988	59
South-east (SE)	165	888	58
South-west (SW)	85	983	90
North-west (NW)	57	1,180	181

The importance of urban dust as a direct source of lead for humans must be assessed in relation to the intake from other sources, and the "provisional tolerable intake" recommended by the WHO. Considering only food, water and air as sources, ingested lead is estimated normally to contribute over 90% of the daily lead intake². Although less efficiently absorbed than inhaled lead⁶, ingested lead is by far the more important source^{5,7}. The recent *Survey of Lead in Food* (United Kingdom)⁸ estimates that for the average adult the intake of lead from food is $\sim 200 \mu\text{g d}^{-1}$, with a further $20 \mu\text{g}$ from liquids. A figure was not derived for children, but on the basis of the recommended caloric intake of food⁹, children (averaged over the ages 1 to 9) would reach 75% of the adult figure, $165 \mu\text{g d}^{-1}$.

A "tolerable intake" of ingested lead for adults was recently established (ref. 9 page 19, and ref. 10) at 3 mg per week (0.05 mg per kg body weight), equivalent to an average daily rate of $430 \mu\text{g}$, infants and children being specifically excluded. But the average weight of children (age 1–9) is $\sim 30\%$ of the adult average⁹, and on a weight ratio a tolerable daily intake would be $130 \mu\text{g}$. If children are more at risk than adults¹¹, this figure should presumably be reduced, and in any case will depend on age.

We conclude, therefore, that although adults on average ingest in food and drink only $\sim 50\%$ of their "tolerable intake", children ingest an amount close to or even above their tolerable level.

For adults, urban dust seems unlikely to contribute a significant quantity of lead to the daily intake. But for children even small quantities of dust would be significant. It seems to us that children who play in areas containing this type of dust are likely to ingest some of it, perhaps by casually eating sweets while playing in the street. We are not aware of any large scale determination of the amount of dust and dirt which is typically ingested by children. We have, however, made a small number of direct measurements and find that a child can transfer from 5–50 mg of dirt from his hands (dirty from 30 min of activity in a normal urban playground) to a typical 'sticky sweet' (5 g). An ingestion of $100 \mu\text{g}$ of lead (contained in 100 mg of average

dust) could result from the consumption, under these conditions, of from 2 to 20 sweets, which probably encompasses children's normal sweet-eating habits. Also, it is possible to envisage lead ingestions much greater than this—20 badly contaminated sweets could contain 1,000 µg of lead.

We conclude that children in urban surroundings, who may already be ingesting in food and drink an amount of lead approaching a tolerable limit, may considerably increase their daily lead intake by the accidental ingestion of dirt and dust from their surroundings in the course of their normal everyday activity. This source of lead does not at present seem to be recognised as a potential hazard. Thus, although upper limits for lead in food (generally 2 p.p.m.) or lead in paint for children's toys (0.5%) are recognised as being desirable, no tolerable upper limit for lead in urban dust has been suggested. We consider that this is an urgent and important problem.

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J. P. DAY
M. HART
M. S. ROBINSON

Department of Chemistry,
University of Manchester,
Manchester M13 9PL, UK

Received May 9; revised October 17, 1974.

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Lead levels in blood of residents near the M6–A38(M) interchange, Birmingham

LEAD levels in blood are generally found to be higher in city dwellers than in those living in the country^{1–6}. This difference has been linked to the increased background levels of lead, which result to a large extent from the emission of lead in motor car exhaust gases. In Birmingham, the opening of the M6–A38(M) interchange provided an opportunity to test this hypothesis by studying the effect of an increase in traffic density on the concentration of lead in the blood of those living close to the interchange.

Capillary blood samples were taken from volunteers living within 800 m of the interchange immediately before its opening in May 1972. Second blood samples were collected between October 1972 and February 1973, and a third collection was made in October 1973. Although a large number of samples were taken, only a relatively small number of persons contributed blood to each of the three series and it is the results from these people which are discussed here. All the estimations of blood lead were carried out at the same laboratory, using an atomic absorption technique. Because of some difficulties with skin contamination experienced during the collection of the first series of blood samples, venous blood was taken for the second and third analyses. No control group was studied.

Two groups were followed through the three series, the first consisting of 41 males and the second of 58 females. The mean

blood lead concentrations in the three series were, for the male group, 14.41, 18.95 and 23.73 µg per 100 ml and for the female group, 10.93, 14.93 and 19.21 µg per 100 ml. For both groups the differences between the first and second, second and third and first and third means are statistically significant as judged by paired *t* test analysis (Table 1).

Table 1 Statistical analysis of mean lead concentrations in the blood of two groups of residents living near the M6–A38(M) interchange

		1:2	Series 2:3	1:3
Males	Mean difference	4.54	4.78	9.32
	Standard error	1.72	1.55	1.46
	<i>P</i>	<0.05	<0.01	<0.001
Females	Mean difference	4.00	4.28	8.28
	Standard error	0.94	0.87	0.95
	<i>P</i>	<0.001	<0.001	<0.001

The M6 opened on May 24, 1972. In April 1972, 361,040 cars a week used the road later incorporated into the interchange. By March 1973, 748,639 cars a week were using the whole interchange and by March 1974 the weekly figure was 900,451.

When interpreting these results due consideration must be given to the fact that the method of blood sampling was changed after the first series and that the samples in each of the three series were not all taken at the same time of year. During the course of the study, however, capillary and venous samples were taken on the same occasion from a total of 96 different individuals in the main study (47 males and 49 females) and it was found that the blood lead concentration in the capillary samples tended to be higher than in the venous samples. Thus any bias introduced into the study by changing the sampling method would seem to be against showing a rise in the blood lead concentration. Similarly, it has been shown that the blood lead concentration is higher in March–July than during the rest of the year⁷ so that in taking the second and third samples during October–February, and not in May when the first sample was collected, a bias is again introduced which is against showing an upward trend. Thus, despite the defects in experimental design which undoubtedly mar the study, the results suggest an increase in the mean blood lead concentration of the two groups of residents over a period when the volume of traffic on the motorway was increasing. The blood lead concentrations are, however, higher than might have been predicted from the atmospheric concentrations using the model of Knelson *et al.*⁸ The concentration of lead in the atmosphere in the residential areas near the motorways is approximately 1 µg m⁻³ although values greater than this are found at sites closer to the road⁹. Knelson and Bridbord¹⁰ have suggested that continued exposure to atmospheric lead levels of the order of 1–3 µg m⁻³ will produce increases in the blood lead concentration, and their theoretical considerations have received some support from experiments on human subjects exposed to low atmospheric lead concentrations produced under controlled conditions¹¹. In these considerations, however, the total amount of lead in the atmosphere is of less importance than its particle size. The model proposed by Knelson⁸ assumes a constant pulmonary absorption rate of 37%. Now if the lead aerosol particles around the interchange are small enough to enable the aerosol to assume the properties of a gas, then maximum lung penetration will be achieved and pulmonary absorption may well be considerably higher than 37%. A preliminary study¹², which has shown that the atmospheric lead levels inside one house near the interchange are almost identical with those outside and follow the same diurnal fluctuation, is consistent with the notion that the aerosol is, in fact, behaving as a gas.

A number of workers^{13–16} have found the mass median diameter of airborne lead particles to be of the order of 0.10–0.90 µm although electron micrographs have shown that

the lead particles may form large aggregates with other material in the atmosphere¹⁷. Whether this aggregation takes place during emission from the tail pipe or during sampling is, however, unresolved. Clearly it would be of considerable interest to have more information about the physical and chemical characteristics which might affect pulmonary absorption of the particles of lead in the atmosphere around the interchange. It should also be borne in mind that factors other than the inhalation of lead from the atmosphere might be contributing to the increase in blood lead—for example, the ingestion of lead-contaminated dust.

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H. A. WALDRON

Department of Social Medicine,
The Medical School,
Birmingham B15 2TJ, UK

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Interactive effects of unpleasant light and unpleasant sound

In tests of colour preference, rhesus monkeys have been found to have a strong aversion to light at the red end of the spectrum^{1,2}. No comparable reaction to colours has been described in healthy human beings although in patients who are suffering from cerebellar or spinal disorders colours may assume a new potency. Thus it has been claimed that in cerebellar patients who exhibit the so-called 'sensorimotor induction syndrome', red light not only exacerbates the motor disorder but disrupts thought processes and leads to acute subjective distress, whereas blue-green light alleviates the symptoms^{3,4}. Furthermore, Halpern and Feinmesser⁵ found that in such patients red light, besides causing discomfort in itself, increases the sensitivity to noxious auditory stimulation: in their experiments the 'threshold of acoustic discomfort' was consistently lowered when the patients wore red filters in front of their eyes. So we have investigated in monkeys whether the aversion to red light is increased when the preference tests are conducted in the presence of unpleasant background noise.

The technique for measuring the monkey's reactions to red light was similar to that previously described². The monkey sat in a small dark chamber with a screen (40 cm × 40 cm) at the far end, on to which red or white light could be projected. The red light (Kodak Wratten filter 25) and the white light were matched in subjective brightness at 1.5 log foot lamberts. The monkey controlled the presentation of the two stimuli by pressing a button. Successive presses

on the button produced the red and the white light in strict alternation, either stimulus staying on for as long as the button was held down. Each test lasted for 400 s of total exposure, during which time the monkeys typically alternated between the two stimuli about 100 times. The relative preference for the red light was measured as the ratio of the time spent with the red light to the total time spent with either red or white light over a defined interval.

The effect of background noise on the colour preference was investigated by comparing the monkey's performance when the chamber was quiet with that when 'white noise' was played through an overhead loudspeaker during the test. The sound pressures in the 'quiet' and the 'noisy' conditions were 59 db and 80 db respectively. For comparisons to be made within a single test, each test was subdivided into eight 50-s bouts, four 'noisy' and four 'quiet' presented alternately. Each monkey was given twenty

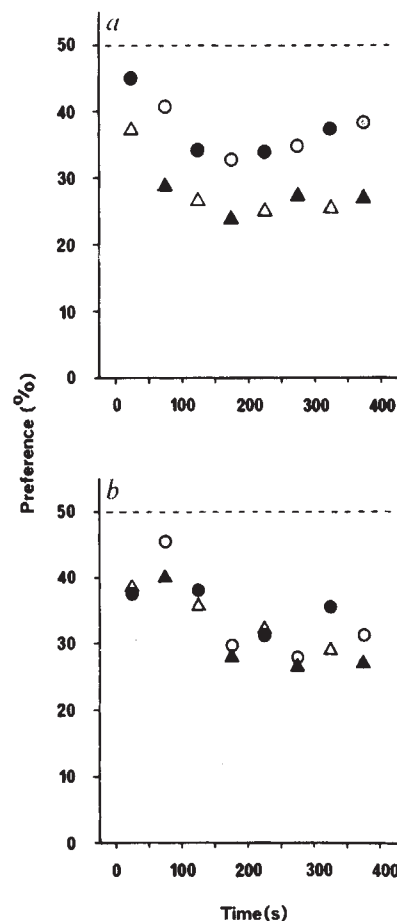


Fig. 1 Preference for red over white light in each successive 50-s period. *a*, Monkeys 1 and 2; *b*, monkeys 3 and 4. ○, ●, 'Quiet' condition; △, ▲, 'noisy' condition. ○, △, Tests which began with noise; ●, ▲, tests which began with quiet. Each point represents the mean of the two monkeys' mean scores over ten tests.

tests, ten of which started with 'noise' and ten with 'quiet'.

Four young adult male monkeys (*Macaca mulatta*) served as subjects. Monkeys are not all equally reactive to white noise and these four were selected as being two who found noise extremely aversive and two who found it relatively tolerable. This assessment was made by running preliminary tests in which the monkeys were given the opportunity to choose between noise and quiet in a simple (noise+light)/(quiet+light) preference test⁶. In five tests, each lasting 400 s, monkeys 1 and 2 preferred the quiet condition 85% and 83% of the time, while monkeys 3 and 4 preferred it 65% and 68% of the time.

The results of the main experiment are shown in Fig. 1,

the two subgroups of monkeys being treated separately. For monkeys 1 and 2 the background noise increased the aversion to red light; for each of these monkeys the effect was significant beyond the 0.001 level (Wilcoxon test, based on the performance within each of the twenty tests). For monkeys 3 and 4, however, the noise had little if any influence; although each of these monkeys showed a marginally greater aversion to red light in the noisy condition, the effect was not significant. The implication is that background noise increases the aversion to red light if, and only if, the monkeys find the noise itself markedly aversive.

Although Halpern and Feinmesser studied the effect of red light on the aversion to noise, whereas we did the reverse in our experiment, there is an obvious parallel between the results of the two studies. The difference in experimental design is in fact more apparent than real, since, whichever way round the experiment is done, the most one can conclude is that a combination of red light and noise is unduly aversive. The experiments do not allow one to distinguish whether it is red light which increases the unpleasantness of noise or noise which increases the unpleasantness of red light; indeed, this is probably a false antithesis, the truth being that the effects of the two stimuli mutually potentiate each other.

Leaving aside the puzzle of why red light should be aversive to either monkeys or cerebellar patients, one may speculate on the broader implications of these findings. Whether it is a general rule that unpleasant stimulation in one modality potentiates the effect of unpleasant stimulation in another remains unknown; but a rule of this sort would be given a certain plausibility by Stevens' 'power law' for subjective sensation⁷. Stevens showed that when people are given electric shocks their subjective discomfort is an accelerating function of the magnitude of the physical stimulus (thus the discomfort resulting from a 40 V shock is more than twice that from a 20 V shock). Suppose, purely for illustrative purposes, that red light alone is equivalent to X units of shock, and noise alone is equivalent to Y units of shock, then the discomfort due to a combination of red light and noise may be expected to be greater than the sum of the discomfort caused by the component stimuli, since $(X+Y)^n > (X^n + Y^n)$, if the sensory exponent, n , is greater than one.

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N. K. HUMPHREY
G. R. KEEBLE

Sub-Department of Animal Behaviour,
University of Cambridge,
Madingley, Cambridge CB3 8AA, UK

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Insect hormones as tsetse abortifacients

FEMALE tsetse flies produce only one offspring at a time, the larva being retained within the uterus where it is nurtured from the female's milk gland¹. The low reproductive potential resulting from this curious form of viviparity is a feature that can perhaps be exploited as a vulnerable link in the life cycle. My experiments show it is possible to disrupt the normal 9 d pregnancy cycle² by using insect hormones to induce abortion.

Glossina morsitans morsitans Westw. was used in the experiments reported here. Females were transferred from

mass culture to individual containers³ shortly before the first larviposition. Flies were offered a blood meal on rabbit ears 6 d a week and feeding records were kept for each fly. The hormones were applied during the second pregnancy cycle; all stages of pregnancy were represented in approximately equal proportions for each dose of hormone applied. Crystals of plant-derived ecdysterone were dissolved in 96% ethanol and diluted with distilled water to 10%; 0.5 μ l was injected into the thoracic tergum by a glass capillary. The juvenile hormone analogue isopropyl (2E,4E)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate (ZR515) was applied to the abdomen in 5 μ l acetone. The flies were held between finger and thumb and were not anaesthetised. Each fly container was examined daily for aborted progeny.

As is seen in Table 1, the effective dose of ecdysterone required to elicit a 50% rate of abortion was between 1.0 and 10.0 μ g. Most abortions occurred during the on-going pregnancy, but a few females aborted again in the next pregnancy. A few aborted only during the next pregnancy. Observations on 45 females kept beyond the second post-injection pregnancy suggest that later pregnancies and female longevity were not affected.

Topical application of the juvenile hormone analogue (JHA) caused a 50% rate of abortion at a dose of approximately 10 μ g. As with ecdysterone, the effect was most pronounced during the on-going cycle (Table 1).

Both hormones induced abortions in the egg stage as well as in all three larval instars. Although the hormones were applied to individuals representing all stages of pregnancy,

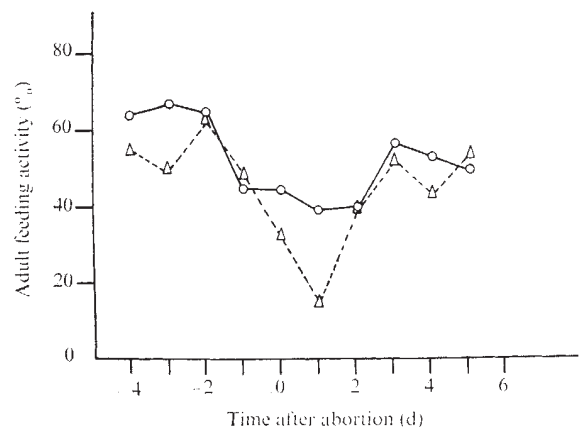


Fig. 1 Incidence of daily feeding activity in tsetse females that aborted following either an ecdysterone injection (○) or a topical application of a juvenile hormone analogue (△). Feeding activity is expressed as the percentage of females that took a blood meal during a 15 min exposure to rabbit ears. Each curve is based on the record of 38-40 flies.

the effect was most frequently manifested in the abortion of 2nd or 3rd instar larvae. The aborted 3rd instars were not fully grown; melanisation of the cuticle⁴ was frequently incomplete and they failed to pupariate. In the case of ecdysterone, 72% of the abortions ($n=43$) were of 3rd instars and 21% of 2nd instars. With JHA 38% of the abortions ($n=42$) were of 3rd instars and 43% were of 2nd instars. The time between hormone application and abortion was much shorter with the JHA: 42% occurred within 24 h and several aborted within 2 h. For abortions occurring during the 1st pregnancy cycle, the latent period was 2.4 ± 0.5 d (mean $\pm$ s.e., $n=36$). In the ecdysterone-treated flies only 7% aborted within 24 h and the mean latent period was 4.2 ± 0.5 d ($n=29$).

All the abortions depressed feeding activity below normal for several days (Fig. 1). For the JHA a pronounced

Table 1 Incidence of abortion in *Glossina morsitans morsitans* Westw. resulting from injection of ecdysterone or topical application of a juvenile hormone analogue

Substance	Treatment Dosage (μ g)	No. of females	Distribution of no. of abortions during two subsequent pregnancies			Females aborting (%)
			1st cycle only	2nd cycle only	1st and 2nd cycle	
10% Ethanol	—	23	0	2	1	13.4
Ecdysterone	0.01	24	0	4	0	16.6
Ecdysterone	0.1	25	3	2	1	24.0
Ecdysterone	1.0	23	5	1	1	30.4
Ecdysterone	10.0	26	17	1	3	80.8
Acetone	—	29	2	0	0	6.9
JHA (ZR515)	0.1	25	1	0	1	8.0
JHA (ZR515)	1.0	25	3	2	0	20.0
JHA (ZR515)	10.0	25	9	1	2	48.0
JHA (ZR515)	50.0	36	13	4	4	79.0

decrease in activity occurred on the day following abortion. In spite of a decline in feeding around the time of abortion, normal larvae were produced in 10.4 ± 1.2 d (mean $\pm$ s.e., $n=25$) after an ecdysterone-induced abortion, and in 9.2 ± 0.6 d ($n=23$) after a JHA-induced abortion.

Effective tsetse abortifacients could have several possible modes of action; first, direct influence on the act of parturition; second, 'turning off' the milk gland; third, disruption of *in utero* larval morphogenesis; or fourth, desynchronisation of the (presumed) female neuroendocrine integration of normal pregnancy².

Larvae within the uterus of a starved female can survive several days before the onset of starvation-induced abortion⁴; therefore it seems unlikely that the rapid abortion initiated by JHA could be the result of effects on the milk gland. As feeding activity in hormone-treated females (Fig. 1) was within the expected range² before abortion, malnutrition is an unlikely cause for the expulsion of immature larvae. Moreover, large milk glands were observed in numerous females that aborted.

Pharmacological agents that act on neuromuscular processes involved in parturition could also potentially act as abortifacients. Physostigmine, a reversible anticholinesterase, was moderately effective as a fast-acting abortifacient. Injection of 0.5 μ l of a 0.1 mM solution of physostigmine (B.D.H. Ltd) in 10% ethanol caused 4 out of 19 females to abort within 15–30 min. All females that did not abort within 30 min successfully completed their pregnancy. Injection of a higher dosage (0.5 μ l of a 1.0 mM solution) caused 100% female mortality. Prostaglandin E₁ and prostaglandin F₂ α -tromethamine salt (Upjohn Co.) injected in dosages of 10 μ g in 1 μ l 10% ethanol were ineffective.

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DAVID L. DENLINGER

The International Centre of
Insect Physiology and Ecology,
PO Box 30772,
Nairobi, Kenya

Received September 27; revised November 25, 1974.

Present address: The Biological Laboratories, Harvard University, 16 Divinity Avenue, Cambridge, Massachusetts 02138.

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Behavioural role of individual components of a multichemical attractant system in the Oriental fruit moth

IN most moths, location of the female for mating is mediated by pheromones¹, which are operationally defined as attractants, although their ability to elicit upwind orientation has been demonstrated in only a few species². Intrinsically unattractive chemicals which modify this process by increasing or decreasing trap catch have been termed synergists and inhibitors¹, respectively, although their behavioural and neurophysiological roles have remained unresolved. In the Oriental fruit moth, *Grapholitha molesta* (Busck), *cis*-8-dodecenyl acetate (*c*8-12:Ac) is a primary pheromone component³ and recently the requirement for attractancy of an isomeric mixture containing about 8% *trans* (*t*8-12:Ac) was demonstrated^{4,5}. Additionally, male trap catch has been reported to be enhanced about two-fold by the simultaneous release of dodecyl alcohol (12:OH) (ref. 5). Specific behavioural functions, however, had not been ascribed to individual attractant components or combinations.

Table 1 Male behaviour within 3 m of a white sticky trap baited with attractant on a rubber septum dispenser

Stimulus	No. of males observed	Percentage captured*	Mean flight time† (s)
100 μ g <i>c</i> 8-12:Ac (6.8% <i>trans</i>)	39	56	19.8
100 μ g <i>c</i> 8-12:Ac (6.8% <i>trans</i>) + 300 μ g 12:OH	40	93	12.5

*Compared with a 2×2 test of independence using the *G* statistic with Yates' correction; $P < 0.01$.

†Means compared with the *t* test; $P < 0.05$. Measurements were initiated on the arrival of males within 3 m of the trap's edge and terminated when males either left this area or were ensnared on a trap's lower sticky surface.

Field studies in Geneva of wild male behaviour near baited traps showed that 100 μ g *c*8-12:Ac alone failed to lure males to the trap vicinity, and indeed no males were observed orienting toward the traps within about 15 m. The combination of *c*8-12:Ac with 6.8% of the *trans* isomer did lure males to the trap (Table 1), and the simultaneous release of 12:OH effected an increase in the number of males actually captured on the sticky trap surface. In terms of trap catch, the attractant 'synergist' (12:OH) seems to increase the frequency of male landings.

Detailed field analyses of the role of 12:OH were carried out using an attractant dispenser placed in the centre of a flat circular table top of radius 60 cm. Previous field trials had revealed that 10 μ g *c*8-12:Ac with 6.8% *trans* elicited the closest mean approach to the dispensers, at doses between 1 and 3,000 μ g. When various quantities of 12:OH were presented along with 10 μ g of the attractant, the presence of 12:OH resulted in significant increases in the frequencies of male

Table 2 Behavioural effects on 12:OH when presented simultaneously with 10 µg of *c*8-12:Ac (6.8% *trans*) on a rubber septum dispenser

12:OH (µg)	No. of males observed	Percentage landing on table top*	Percentage fanning while walking on table top*	Percentage hair-pencil display on table top*	Mean closest approach† to attractant dispenser (cm) ± s.e.
0	37	68	27	3	47.5 ± 2.5
1	40	88‡	73¶	8	33.6§ ± 3.2
3	39	77	36	23‡	36.7‡ ± 3.9
10	33	94¶	83¶	42¶	19.9§ ± 3.9
30	40	80	45	15	35.3¶ ± 3.6
100	30	83	43	30‡	28.5§ ± 4.3

*Percentages in the same column compared with the treatment lacking 12:OH with a 2×2 test of independence using the *G* statistic with Yates' correction.

†Means compared with the treatment lacking 12:OH according to the *t* test. Males flying within 0.5 m of the table top edge but not landing were scored as approaching within 60 cm. The remaining approaches were by males walking on the table top.

‡ $P < 0.05$

¶ $P < 0.01$

§ $P < 0.001$

landings, wing fanning while walking, approach to the chemical stimulus and extrusion of hair-pencils* at the abdominal tip (Table 2). This is the first example of a particular chemical component evoking hair-pencil display.

Although to date only *c*8-12:Ac has been confirmed from female *G. molesta*, this species seems to utilise a multicomponent communication system. Both *c*8- and *t*8-12:Ac are requisites for attraction but this combination seems to elicit relatively little fanning and almost no extrusion of hair-pencils close to the attractant source. The main effect of 12:OH, previously considered an attractant synergist, is apparently to elicit a repertoire of precopulatory behaviour, which is likely to increase the catch in a trap.

The finding of disparate communicative functions for chemicals thought to be attractants and modifiers has implications for control programmes based on the disruption technique, in which the pheromone permeates the atmosphere so that either the males fail to locate the females (confusion) or habituation of mating responses⁷ occurs. Successful implementation of this procedure may ultimately depend on an intimate understanding of the precise communicative role of individual pheromonal components, since manipulation of attraction alone may not obviate mating.

R. T. CARDE
T. C. BAKER
W. L. ROELOFS

Department of Entomology,
New York State Agricultural Experiment Station,
(Cornell University), Geneva, New York 14456

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Coral diseases in Bermuda

WE report here diseases of reef corals that seem to be associated with bacterial infection. On reefs around Bermuda, where our field work was carried out in the summer of 1973, the most commonly affected species are the brain corals *Diploria labyrinthiformis* and *D. strigosa* (Fig. 1). We believe that this is the first unequivocal report of a coral disease and our observations suggest that diseases may be important agents of coral death. (There are some indications that others have seen diseased corals^{1,2}.)

The distribution of diseased colonies is difficult to evaluate because only 0.5–1% of the *Diploria* colonies are affected at a given time. Diseased colonies, however, occur throughout the reefs. Their distribution seems unrelated to inshore

pollution sources and to areas of greater or lesser circulation.

On the reefs the *Diploria* disease appears as a dark brown line separating a dead from a live portion of a colony. The coral is apparently healthy, with all its zooxanthellae up to the disease line (Fig. 2), while on the other side of the line no tissue remains. The disease consumes tissue at a rate of a few centimetres per month. The exposed corallum behind the line of infection is soon covered with filamentous algae and diatoms.



Fig. 1 Infected colony of the brain coral *Diploria strigosa* in 5 m of water at North Rock, Bermuda. The infected area, with black disease line and exposed white corallum, is at the lower left.

The disease seems to initiate at the margins of living colonies, or at irregularities such as areas of boring bivalves or barnacles, and to spread outwards from such points. We have seen no evidence for the initiation of the disease in the middle of healthy tissue. The disease does not always kill an entire colony, but rather dies out itself in time, leaving colonies with dead patches and living areas, the latter raised up by their continued growth.

Microscopic examination showed the disease to be a dense mat of bacterial filaments in which diatoms, ciliates and released zooxanthellae are embedded. The principal organism is the gliding bacterium *Beggiatoa*, a heterotroph requiring a source of sulphide such as H_2S to initiate its catabolic activities³.

By exposing healthy *Diploria* colonies to reducing conditions in aquaria, we have been able to foster *Beggiatoa*

infestations. In our experiments, reducing conditions over the coral surface were exploited by the sulphate-reducing anaerobic bacterium *Desulfovibrio*, which produced copious amounts of H_2S gas. Then *Beggiatoa* colonised the air- H_2S interface and attacked the coral tissue.

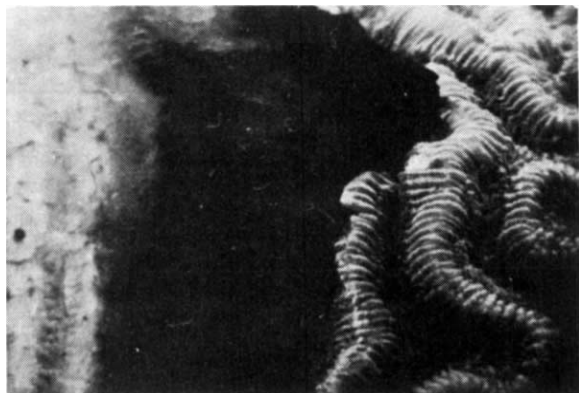


Fig. 2 Close-up view of the disease line on *D. strigosa*. Healthy tissue on right. The disease line is approximately 15 mm wide.

Such artificial infestation has not produced the localised dark line disease, but we find *Desulfovibrio* and *Beggiatoa* in the natural as well as in the artificial infections. The involvement of these microorganisms in both types of disease may be related only to decay (that is, the end stage of the disease). The close proximity of the *Beggiatoa* mat to healthy tissue in the natural disease, however, suggests its involvement at least in the spreading, and possibly in the initiation of the disease.

We have also occasionally observed the dark line disease on *Montastrea annularis*, and a disease net, similar to our artificially induced disease, on *Porites astreoides*. It seems likely that such diseases attack other corals in other reef areas, although because of the short life span of the disease (weeks to months) the numbers of diseased colonies will always be small. The large number of coral colonies on the reefs of Bermuda with dead patches, many of which could be disease-related, indicate that disease may be an important factor in coral ecology.

PETER GARRETT

Bermuda Biological Station,
St George's West, Bermuda

HUGH DUCKLOW

Engineering Sciences Laboratory,
Harvard University,
Cambridge, Massachusetts 02138

Received September 3; revised November 27, 1974.

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in association with callus cultures of non-legumes as well as with cultures of legume species which do not form nodules with the cowpea bacteria.

Three rhizobial strains were tested for contamination by non-symbiotic dinitrogen-fixing bacteria and maintained on a medium containing K_2HPO_4 0.5 g; $MgSO_4 \cdot 7H_2O$ 0.2 g; NaCl 0.1 g; yeast extract 0.5 g; $CaCO_3$ 0.5 g; mannitol 10 g l^{-1} water. Tissue cultures of brome grass (*Bromus inermis* Leyss.), wheat (*Triticum monococcum* L.), rapeseed (*Brassica napus* L.), sweet clover (*Melilotus alba* Desr.) and *Vicia hajastana* (Prairie Regional Laboratory⁵) were routinely examined by light microscopy, and subcultured on nutrient agar and in nitrogen-free media³. These tests confirmed the absence of microbial contaminants. Nitrogenase activity was established essentially as described for the soybean-*R. japonicum* combination⁶. Plant cells from suspension cultures were placed on a membrane lying on top of 50 ml low-nitrogen nutrient agar. After callus growth was established (7-20 d) the surface was infected with a small volume of *Rhizobium*. After a further 7-14 d, the vessel was sealed and endogenous ethylene production measured by gas chromatography after 1 h. Acetylene was injected, and after an hour another sample of the gas phase removed for analysis. The reduction of acetylene to ethylene is a positive indication of nitrogenase activity⁷. The plant cells were removed from the membrane for microscopic examination and dry weight determination.

Rhizobium sp. 'cowpea' 32H1 formed nitrogenase (C_2H_2) in association with plant cells of five field pea varieties, two soybean varieties, sweet clover and *Vicia hajastana*, as well as the non-legumes rapeseed, wheat and brome grass (Table 1). The possibility of infection by non-symbiotic dinitrogen fixing bacteria was excluded by subculturing positive callus-rhizobial associations on to yeast extract-mannitol agar, glucose-peptone agar and *Azotobacter* media³. There was no growth on glucose-peptone or nitrogen-free nutrient, and colonies observed on yeast extract-mannitol agar were microscopically indistinguishable from cowpea rhizobia. The dinitrogen fixing association described is aerobic^{6,8} and it is unlikely that it is due to contamination by *Klebsiella*, which usually only fixes anaerobically. The nutrient used⁶ has a low pH (5.5), and is unlikely to permit

Table 1 Acetylene reduction activity of associations of *Rhizobium* sp. 32H1 and various plant callus cultures

	Plant cells + <i>R. sp. 32H1</i>	Specific activity (nmol C_2H_4 h^{-1} g^{-1} dry weight) uninfected plant cells
Pea var. 'Century'	530	1.7
'Trapper'	1,250	8.8
MP766	109	2.5
MP7608	251	2.1
EMD	413	5.7
Soybean var. 'Acme'	353	—
'Mandarin'	7.3	—
<i>Vicia hajastana</i>	335	0.9
Sweet clover	108	3.6
Rapeseed	131	5.7
Wheat	83	0.6
Brome grass	323	0.2

Nitrogenase activity was determined by the reduction of C_2H_2 to C_2H_4 , followed by determination of the dry weight of callus. Each value is the mean of five samples. Rhizobial strains were obtained from Dr J. C. Burton, the Nitragin Company, 2101 West Custer Ave, Milwaukee, Wisconsin 53209, USA. Strains 32H1 and 32Z3 were isolated from *Crotalaria* and strain 21A6 was isolated from *Chamaerista fasciculata*. Single colony isolates were prepared from dilution plates. All strains are motile Gram-negative rods, produced effective nodules on cowpea plants, grew well on yeast extract-mannitol agar, and formed uniform pale colonies on yeast-mannitol-congo red agar. The three strains formed a slight 'serum zone' in litmus milk after 10-14 d incubation². There was no growth on glucose-peptone agar, sporulation agar or several standard nitrogen-free media³. Absence of *Klebsiella* was indicated by the lack of growth, acid or gas formation in anaerobic semi-solid peptone-carbohydrate media⁴.

Nitrogen fixation by a *Rhizobium* sp. in association with non-leguminous plant cell cultures

THE genus *Rhizobium* is characterised by its ability to elicit nodule formation and fix dinitrogen in roots of legumes and its species or groups are classified according to their legume host range. There is only one non-legume association reported, that of *Trema aspera* with a 'cowpea-type' strain of *Rhizobium*¹. Dinitrogen fixation by *Rhizobium* in the absence of a host plant has not yet been demonstrated. I report the production of nitrogenase (C_2H_2) activity in *Rhizobium* sp. 'cowpea strain' growing

dinitrogen fixation by *Azotobacter*, which usually prefer an almost neutral pH.

Examination by light or electron microscopy showed that rhizobia grew on the surface of the callus and in cracks and intercellular spaces. Where bacteria were found in plant cells, destructive invasion had occurred. Moreover, the few such infected plant cells could not account for the observed acetylene reduction. In no case was a plant cell containing rhizobia observed which looked like a normal nodule cell. The bacteria always appear as rods rather than bacterioids, and no membrane surrounding the bacteria could be detected. The *Rhizobium*-plant callus association differs from the nodule system in its morphology and its reaction to plant growth regulators^{6,8}. A nodule-like infection process does not seem to be necessary to establish nitrogenase activity in the tissue culture system.

After the membranes were lifted to remove the callus for examination, a copious growth of rhizobia was often seen on the agar. The vessels were resealed, and acetylene again added. After an hour, ethylene production was measured, and it was found that 5–25% of the nitrogenase activity of the original systems could be accounted for by these bacterial cells not in direct contact with plant callus. The nitrogenase activity continues for 24 h, but does not persist when these rhizobia are transferred to nitrogen-free media.

Table 2 Acetylene reduction activity of associations of three strains of 'cowpea' *Rhizobium* and various callus cultures

<i>Rhizobia</i> sp. Plant cell line:	Specific activity (nmol C ₂ H ₄ per h per g dry weight)		
	32H1	32Z3	21A6
Soybean var. 'Acme'	105	56	0
<i>Vicia hajastana</i>	178	26.5	23.9
Sweet clover	114	9.0	7.0
Rapeseed	205	19.0	20.6

Nitrogenase activity determined as in Table 1.

The ability to form nitrogenase in the presence of non-host plant cells was also found in three cowpea strains (Table 2). Nitrogenase activity was, however, not obtained from *R. japonicum* or *R. leguminosarum* in the presence of non-legume tissue cultures.

Phillips⁹ has reported nitrogenase activity in an association of soybean cells and cowpea strain 32H1. This was not surprising, as *R. japonicum* and the cowpea groups are closely related, both belonging to the 'slow growing' rhizobia, and cross inoculation between soybean and cowpea has been found⁴. Clover, pea and *Vicia* are nodulated by the 'fast growing' group of *Rhizobia* and nodulation of intact plants by cowpea *Rhizobium* is unknown².

The genes coding for nitrogenase are found in the bacteria^{10,11}. What elicits the expression of nitrogenase activity is unknown but the diffusible factor(s) involved are obviously not limited to legumes. The demonstration of nitrogenase in the presence of legume, rapeseed, wheat and brome grass cells suggests that some common plant product may be involved. It is likely therefore that in symbiotic dinitrogen fixation the species barriers are at the stages of infection and nodule formation, and not in the expression of nitrogenase.

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J. J. CHILD

Prairie Regional Laboratory,
National Research Council of Canada,
Saskatoon, Saskatchewan, Canada S7N 0W9

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Nitrogen fixation by *Rhizobium* associated with tobacco and cowpea cell cultures

THE maxim that nitrogen fixation by the root nodule bacteria, *Rhizobium*, is restricted to a formal symbiotic association with specific legumes has recently been challenged. Trinick¹ showed that nodules formed on the non-legume *Trema canabina* (previously identified as *T. aspera*; M. J. Trinick, personal communication) by a strain of *Rhizobium* which nodulated *Vigna unguiculata* (cowpea), possess nitrogenase activity and fix atmospheric nitrogen. Soybean tissue cultures inoculated with *R. japonicum*^{2–4}, or with cowpea strains of rhizobia⁵, also possess apparently functional nitrogenase as determined by the acetylene reduction assay⁵. Several attempts have failed to demonstrate nitrogenase activity in cultured rhizobia⁶, including cowpea strains⁷.

We report nitrogenase-mediated acetylene (C₂H₂) reduction, and ¹⁵N incorporation, in associations of a strain of cowpea rhizobia (32H1) with cell cultures of a natural host, *V. unguiculata*, but more notably with a non-legume, *Nicotiana tabacum*. Furthermore, this strain of rhizobia reduced C₂H₂ when cultured adjacent to, but not in contact with, tobacco callus.

Cell cultures, derived from stem explants of *N. tabacum* cv. Wisconsin 38 and *V. unguiculata* cv. Poona, were maintained as 30-ml suspensions in Schenk and Hildebrandt⁸ (SH) medium with 2,4-dichlorophenoxyacetic acid (9 μM) and kinetin (0.2 μM) in 250 ml flasks on a gyratory shaker (100 r.p.m.) at 29°C in the dark. A culture of strain 32H1 (J. C. Burton, Nitragin Co., Milwaukee, Wisconsin⁹) was checked for purity by two successive isolations of single colonies on yeast-mannitol agar. Actively growing plant cell suspensions were inoculated with 2 × 10⁸ bacteria (1 ml), shaken for 48 h, centrifuged, and washed in cell culture medium. Aliquots (0.8 g fresh weight) of the pellet were spread on filter paper, drained of excess liquid and the cells and filter paper incubated on the surface

Table 1 Acetylene-dependent ethylene production* by 32H1-plant cell associations

	<i>N. tabacum</i> (nmol C ₂ H ₄ per g dry weight per hour)	<i>V. unguiculata</i> (nmol C ₂ H ₄ per g dry weight per hour)
GLN medium†	106 ± 21	7 ± 2
RN medium‡	118 ± 8	6 ± 2

*Cultures were sealed with serum stoppers and assayed after 2 h for endogenous ethylene (C₂H₄) which was found only in uninoculated tobacco cells (<7 nmol g dry weight⁻¹ h⁻¹). The gas phase was replaced with a mixture of 20% O₂: 20% C₂H₂: 60% argon and after 2 h, the reduction of C₂H₂ to C₂H₄ was measured by gas chromatography. Acetylene reducing activity (nmol C₂H₄ per g dry weight per hour) was corrected for endogenous C₂H₄ production where applicable. The limit of resolution was 0.5 nmol per g dry weight per hour in 2 h assays.

†SH medium⁸ without phytohormones and with major salts replaced by (in mM) KH₂PO₄ (2.2), CaCl₂·2H₂O (0.7), KCl (0.9) and MgSO₄·7H₂O (0.3) and with decreased inositol (0.6) and added glutamine (10) and glycine (0.03); 10 ml media in 18 ml bottles.

‡SH medium without phytohormones and with KNO₃ and NH₄H₂PO₄ replaced by (in mM) NH₄NO₃ (1.5) and K₂HPO₄ (2.9) plus added glucose (50); 12 ml media in 28 ml bottles.

of plant culture media slants at 29° C in the dark. These and control cell cultures were assayed for acetylene-dependent ethylene production (Table 1) after 10 d.

Nitrogenase activity, indicated by C_2H_2 reduction, was appreciable in the 32H1–tobacco cell associations, and low but positive in the 32H1–cowpea cultures (Table 1). There was no C_2H_2 reduction by control plant cells or by 32H1 cultured alone on RN or GLN media, or by 32H1 on yeast–mannitol agar. The mean activity of all inoculated tobacco cultures was comparable to the activity reported for *Rhizobium*–soybean associations^{2–4}, but there was less variability under our conditions. The rate of C_2H_2 reduction by six inoculated tobacco cultures on RN medium was linear for 6 h but declined by 40% after 20 h; activity in the two other replicates declined after 2 h.

There was copious growth of rhizobia in inoculated tobacco cultures and on the exposed and underlying surface of the agar slants. When the filter paper with inoculated cells was removed and discarded, the residual rhizobia on the slant maintained nitrogenase activity for 8 h at 2.2 nmol ethylene per culture per hour. This was 16% of the average value for undisturbed cultures.

The indication that the rhizobia possessed nitrogenase activity, and that activity depended on the presence of plant cells, was confirmed by growing a lawn of 32H1 on a GLN agar plate to within 5 mm of uninoculated tobacco callus. Bacterial growth was enhanced close to the tobacco callus, and after 7 d, agar segments (2.3 cm²) containing only bacteria, only callus, or neither, were excised and assayed. The segments with rhizobia reduced C_2H_2 at 0.8 nmol per segment per hour and activity was linear for 8 h, declining to 60% after 60 h. No activity was found in the other segments or in 32H1 cultured in the absence of plant cells.

Fourteen day old 32H1–tobacco cell associations on GLN agar slants were exposed to an atmosphere of 20% O₂ and 80% N₂, containing 80 atoms per cent ¹⁵N, for 28 or 42 h. The inoculated cells on filter paper and the underlying bacteria from the agar were removed separately, and ¹⁵N enrichment of the samples determined by mass spectrometry. The values for the cells were 0.034 and 0.039 (28 h) and 0.048 (42 h), while the corresponding values for the bacteria were 0.461, 0.612 and 0.483 atoms per cent excess compared with values of zero for identical material incubated in air. These highly significant ¹⁵N enrichments conclusively verify that the C_2H_2 –reducing activity observed in these experiments was due to nitrogenase.

The interest of these results depends on the evidence that our 32H1 culture was an uncontaminated strain of *Rhizobium* and that the plant cell suspensions were uncontaminated. Our culture of 32H1, initially purified by two successive single colony isolations, was slow-growing on yeast–mannitol agar and was both culturally and microscopically (Gram negative, motile rods) uniform. There was no growth, aerobically or anaerobically under N₂, on N-free or on yeast–peptone media, whereas *Klebsiella pneumoniae*, M5al, grew rapidly under these conditions. Cowpeas growing in sterilised vermiculite–perlite were inoculated with a suspension of 10⁸ bacteria ml⁻¹, or a 10⁷ dilution of this, and produced nodules within 7 and 14 days, respectively; mature nodules reduced C_2H_2 . Bacteria reisolated from nodules were uniform, culturally identical to the parental 32H1, and formed C_2H_2 –reducing associations with tobacco cells. We verified the homogeneity of our 32H1 culture by producing both C_2H_2 –reducing associations with tobacco cells and nodules on cowpea with each of 15 single colonies isolated from the original culture; these isolates, cultured alone on GLN medium, did not reduce C_2H_2 . Only slow-growing bacteria similar to our parental 32H1 were recovered from each of the 15 inoculated tobacco cell suspensions. Uninoculated tobacco cell suspensions, cultured on yeast–mannitol, yeast–peptone, or under N₂ on N-free media, showed no evidence of bacterial contamination. We are confident that our 32H1 culture was a pure strain of

Rhizobium and that the tobacco suspensions were uncontaminated.

Our results indicate that the genetic information for nitrogenase is encoded in *Rhizobium*, as suggested by other evidence^{7,10,11}. Expression of the nitrogenase gene appears to depend on a diffusible factor secreted by plant cells.

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W. R. SCOWCROFT
A. H. GIBSON

Division of Plant Industry,
CSIRO, Canberra, Australia

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An attempt to separate fractions rich in human Y sperm

THE desire to predetermine sex before conception and thus alter the natural sex ratio has led many investigators to seek methods of separating the populations of X- and Y-bearing sperm and over the past 50 years, a number of claims of success have been made. Ericsson *et al.*¹ described a technique for the isolation of fractions rich in human Y sperm. On repeating the methods outlined in their paper, we were unable to find any evidence of enrichment of human Y sperm.

Initially, human semen was obtained from a hospital clinic, but there were two disadvantages; first, many of the samples showed poor sperm density and motility, and second, the time between ejaculation and reception of the semen at the laboratory was very variable. Later, an arrangement was made for seven anonymous volunteers to provide normal semen which could be used within 3–4 h of ejaculation. On arrival, all semen samples were examined on a warmed stage under a light microscope and assessed for percentage motility and degree of progressive motility. With a reasonable sample, 70–75% sperm showed progressive motility initially.

The separation technique was essentially that described previously¹ except that the fractions were obtained from the

Table 1 One-layer technique

Experiment no.	Normal semen smear (control)			Sperm from 6% BSA fraction		
	Total no. of sperm counted	No. of Y sperm counted	% Y sperm	Total no. of sperm counted	No. of Y sperm counted	% Y sperm
1	322	106	32.9	306	99	32.4
2	306	123	40.2	413	141	34.1
3	309	121	39.2	541	203	37.5
4	366	113	30.9	423	113	26.7
5	320	94	29.4	422	110	26.1

Table 2 Two-layer technique

Experiment no.	Normal semen smear (control)			Sperm from 6% BSA fraction			Sperm from 15% BSA fraction		
	Total no. of sperm counted	No. of Y sperm counted	Y sperm (%)	Total no. of sperm counted	No. of Y sperm counted	Y sperm (%)	Total no. of sperm counted	No. of Y sperm counted	Y sperm (%)
6	126	46	36.5	121	52	43.0	43	18	41.8
7	413	143	34.6	200	72	36.5	155	56	36.1
8	120	51	42.5	299	111	37.1	155	47	30.3
9	210	79	37.6	209	84	40.2	200	85	42.5
10	209	83	39.7	218	64	29.4	104	35	33.7
11	221	99	44.8	212	84	39.6	214	72	33.6
12	313	153	48.9	129	50	38.8	183	65	35.5
13	504	200	39.7	629	150	23.8	593	150	25.3
14	618	254	41.1	333	101	30.3	250	56	22.4

bottom of the column. The semen was prepared by diluting 1:1 (v/v) with Tyrode's solution, centrifuging at 2,500*g* for 15 min and then resuspending the sperm in Tyrode's solution to give a final sperm density of approximately $100 \times 10^6 \text{ ml}^{-1}$. The semen was kept at room temperature throughout.

Columns of bovine serum albumin (in Tyrode's solution) were prepared in Pasteur pipettes (internal diameter 5.7 mm), closed by a clip on soft polythene tubing at the point of tapering. By controlling the clip, the flow of liquid from the column was easily regulated. Three separation procedures were used.

(1) One-layer technique. Each column contained 0.9 ml bovine serum albumin (BSA) in Tyrode's solution, of set concentration—a 6% solution was used in this series of experiments. The resuspended sperm (0.5 ml) (approximately 50×10^6 sperm) in Tyrode's solution were layered carefully on top of the albumin solution and left for 1 h. The top sperm layer was then removed with a pipette and the lower albumin fraction (isolation fraction) was removed from the base of the column. The sperm were recovered from the albumin by centrifugation at 2,500*g* and washed once in Tyrode's. The sperm were then resuspended in a little Tyrode's solution and smears made for staining.

(2) Two-layer technique. This was the modification described by Ericsson whereby discontinuous gradients of albumin solutions were prepared within the same Pasteur pipette column. Fifteen per cent BSA solution (0.4 ml) was overlaid with 0.8 ml 6% BSA solution. Sperm, prepared and resuspended in Tyrode's as before, was applied carefully to the top of the column. The column was left for 1 h, then the top sperm layer was pipetted off and the column was left for a further 30 min before the separate albumin layers were drawn off slowly from the base of the column. The separate BSA fractions were centrifuged at 2,500*g* and the sperm washed in Tyrode's solution (usually only one washing) before smears were made for staining.

All smears were air-dried and fixed in methanol for 30 min. The smears were stained with 0.5% aqueous solution of quinacrine dihydrochloride (Atebrin, Gurr) for 8 min followed by a brief tapwater rinse and a brief deionised water rinse, before mounting in water with a sealed coverslip. The smears were examined under oil with a dark field condenser at $\times 1,000$ magnification, using a Leitz microscope with a mercury vapour lamp and BG12 and 38 exciter filters. The total number of sperm counted was around 200 per sample which is the number used in our routine procedure for assessment of sperm quality and morphology. The proportion of these with a fluorescent spot or F body within the sperm head was noted. In a study of this nature, the staining procedure is of paramount importance. We investigated the effect of the length of time on staining and found that 8 min gave us the most consistent results. The assessment of the Y sperm was done very critically in that if there was any doubt at all as to the presence of an F body, it was assumed to be absent. Such rigorous assessment of the Y sperm would undoubtedly lower the apparent percentage present.

As an experimental control, smears were made of untreated semen before preparing the sample for column separation. These smears were fixed, stained and examined at the same time as the experimental smears. We also investigated the effect of BSA on the staining process and in our hands we found that the presence of BSA did not affect the staining and difficulty was only encountered when we attempted to use 20% BSA.

Tables 1–3 show the results of 19 experiments in which a total of 14,374 sperm were counted. The presence of a fluorescent spot within the sperm head was taken to denote a Y-bearing sperm.

As can be seen from the tables of results, no positive increase in the proportion of Y sperm was achieved by passing the sperm through columns of BSA solutions. It might seem from the results that there is an increase in the number of X sperm. This is most likely because of the rigorous criteria which we applied to

Table 3 Three-layer technique

Experiment no.	Normal semen smear (control)			Sperm from 6% BSA fraction			Sperm from 10% BSA fraction			Sperm from 15% BSA fraction			Sperm from 20% BSA fraction		
	Total no. of sperm counted	No. of Y sperm counted	Y sperm (%)	Total no. of sperm counted	No. of Y sperm counted	Y sperm (%)	Total no. of sperm counted	No. of Y sperm counted	Y sperm (%)	Total no. of sperm counted	No. of Y sperm counted	Y sperm (%)	Total no. of sperm counted	No. of Y sperm counted	Y sperm (%)
15	238	93	39.0	205	72	35.1	—	—	—	209	106	50.7	154	67	43.5
16	135	56	41.5	60	30	50.0	—	—	—	150	30	20.0	105	28	26.7
17	209	81	38.8	—	—	—	64	29	45.3	108	40	37.0	20	6	30.0
18	210	67	31.9	—	—	—	115	17	14.8	288	89	30.9	190	66	34.7
19	316	110	34	—	—	—	328	85	25.9	288	71	21.7	273	78	28.6

(3) Three-layer technique. The column was prepared by layering decreasing gradients of 20%, 15% and 10% or 6% BSA solutions over one another in 0.3 ml volumes. The top layer was 0.5 ml resuspended sperm in Tyrode's solution. The column was left for 1 h before the top layer was pipetted off and then for a further 60 min before the albumin fractions were drawn off. The sperm were removed from the separate BSA fractions by centrifugation at 2,500*g* and usually washed once in Tyrode's solution before smears were made for staining.

the identification of the Y sperm and does not indicate an actual increase in the number of X sperm.

Separation is dependent on the progressive motility of the sperm. We encountered difficulty in resuspending the sperm evenly after centrifugation. The process of washing the sperm and removing the seminal plasma resulted in a reduction in the percentage of progressively motile sperm in the first layer of the column. Clumps of sperm were formed which were so large that they could be seen to fall through the albumin layers under

gravity. The multiple layer techniques seemed to be preferable to the one-layer procedure because sperm were not subjected to centrifugation and resuspension before being placed on further albumin columns to complete the separation.

We have been unable to confirm the results obtained by Ericsson *et al.*¹ The crucial parts of the procedure are the staining process and the subsequent identification of Y sperm. The standardisation of our staining technique and the criteria which we applied to the identification of the Y sperm are not responsible in our opinion for the negative results which we have obtained.

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J. M. EVANS
T. A. DOUGLAS*
J. P. RENTON

Departments of Veterinary Clinical Biochemistry (Hospital) and Reproduction,*

*University of Glasgow Veterinary School,
Bearsden Road, Bearsden, Glasgow G61 1QH, UK*

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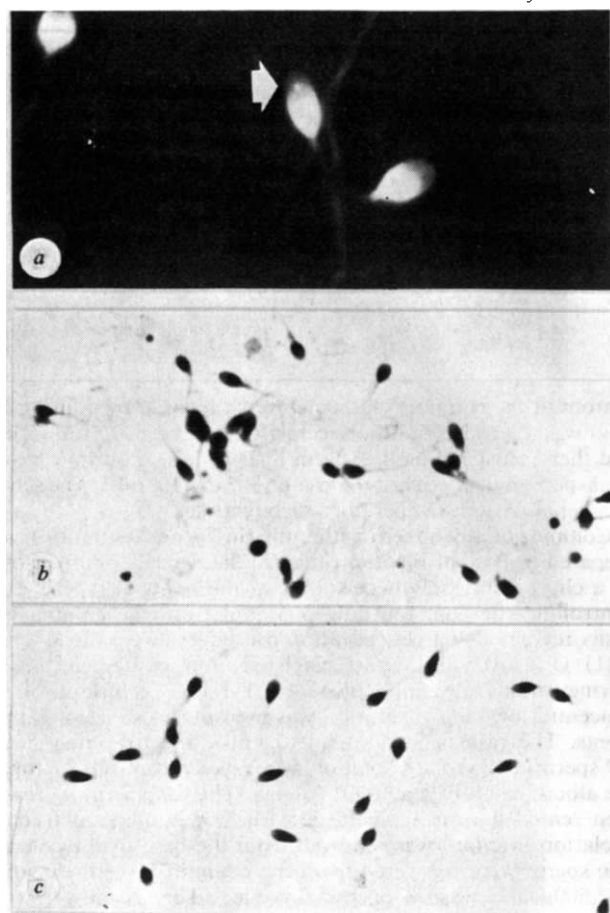


Fig. 1 *a*, Quinacrine stained spermatozoa showing the presence (arrowed) or absence of the fluorescent Y body. *b*, Spermatozoa from sample 5 before layering over BSA, showing the heterogeneous morphology of the sperm heads. *c*, Spermatozoa from sample 5 after layering over BSA (final isolate) showing a homogeneous morphology of sperm heads.

Failure to confirm separation of X- and Y-bearing human sperm using BSA gradients

THE ability to predetermine the sex of offspring before conception would have significant clinical and sociological implications in man¹ and would have great economic benefit if applied to the breeding of livestock. The claim of Ericsson *et al.*² that human spermatozoa bearing Y chromosomes can be separated from sperm containing X chromosomes by passage through a medium containing bovine serum albumin (BSA) or ovalbumin, is therefore of some importance. We have attempted to repeat the experiments to confirm these findings and report here our failure to do so.

The experiments of Ericsson *et al.*² yield two important conclusions. First that it is possible to separate the more motile fraction of human spermatozoa by layering a suspension of sperm in Tyrode's solution over various concentrations of BSA in single or multiple steps, when the more motile sperm enter the layers of higher concentration while the less motile sperm are retained within the Tyrode's solution or the less dense layers. Second, that sperm selected for increased motility in this way turn out to be predominantly Y-bearing sperm: for example in two experiments where sperm motility was

increased from around 65% to 98%, the percentage of Y-bearing sperm was reported to have increased from around 50% to 80%. In these experiments, the sex chromosome status of spermatozoa was ascertained by scoring for the presence or absence of a brightly fluorescing Y body (equated with the Y chromosome, see Fig. 1*a*) in samples stained with quinacrine dihydrochloride³⁻⁵. Our work confirms the first conclusion but not the second.

A series of initial experiments was performed to test the effects of various concentrations of BSA and of different fixation procedures which might influence the fluorescent staining and Y-body detection in spermatozoa. It was found

Table 1 Sperm motility (%) and morphology*

Individual (Sample no.)	Control untreated (a)	Washed in Tyrode's and centrifuged (b)	Top layer	10% BSA	15% BSA	25% BSA
1	50 (25)	30	1			80 (50)
2	40 (—)	20	10			70 (—)
3	70 (67)	40	40	1	10	50 (77)
4	80 (51)	20	2	5	12	80 (78)
5	60 (51)	60	2	7	70	90 (68)
6	80 (64)	80	2	30	80	100 (84)
7	30 (22)	30	1	2	25	75 (88)
8	55 (20)	50	4	7	20	89 (94)

* Figures in parentheses are estimates (%) of sperm with normal morphology based on 100 spermatozoa per sample: the remaining entries are data on sperm motility based on a minimum of 100 sperm per sample.

The difference in motility (%) between (a) and (b) in samples 1-4 is the result of centrifugation in Tyrode's at 1,750g. There is no difference in motility (%) between sperm in untreated semen and samples washed and centrifuged at 800g, compare (a) and (b) for samples 5-8.

Table 2 Percentage of sperm with Y bodies

Individual (Sample no.)	Washed sperm Control		Top layer		10 %BSA		15% BSA		25 % BSA	
	No. of sperm counted	Y sperm (%)	No. of sperm counted	Y sperm (%)	No. of sperm counted	Y sperm (%)	No. of sperm counted	Y sperm (%)	No. of sperm counted	Y sperm (%)
1*	200	49	200	43					200	41
2*	200	47	200	51					200	44
3	200	45	200	40	200	57	200	49	200	48
4	200	45	200	48	200	48	200	45	200	44
5	200	41	200	48	200	45	200	31	200	46
6	400	49	400	51	400	51	400	51	400	50
7	400	45	400	45	400	45	400	44	400	44
8	400	46	400	49	400	48	400	46	400	46
Total	2,200	46	2,200	47	1,800	49	1,800	45	2,200	46

* The samples from subjects 1 and 2 were subjected to a single-step and the remainder to a three-step isolation procedure.

that the efficiency of detecting a Y body was not influenced by BSA concentrations over a range of 6–25%, although higher background fluorescent levels were observed at the higher concentrations even though the samples had been washed four times in Tyrode's solution. Better fixation and staining was obtained from suspensions of sperm fixed in methanol: acetic acid (3:1) than in methanol alone.

In the experiments reported here, semen samples were obtained from each of five healthy volunteers and three males attending a subfertility clinic. Each sample was subjected to either the simplest single-step, or the most efficient three-step continuous gradient, described by Ericsson *et al.*². In all cases the sperm count, volume and motility were estimated on the fresh specimen and a smear was prepared for morphological studies. The remainder of the sample was washed in Tyrode's solution (1:1) and centrifuged at 4,000 r.p.m. (1,750g) for 15 min and resuspended in Tyrode's solution at a concentration of 1×10^8 sperm ml⁻¹.

In the first four specimens studied it was noted that sperm motility was reduced after washing and centrifugation at 1,750g (see Table 1) and further experiments were carried out to examine possible effects of centrifugation on sperm motility in Tyrode's solution. It was found that 800g (2,000 r.p.m.) was the maximum centrifugal force with no adverse effect on sperm motility in Tyrode's solution and this was used in the later experiments. This effect on motility was only observed after centrifugation of sperm in Tyrode's solution and was not apparent when sperm were centrifuged in BSA/Tyrode's solutions.

Single-step gradients were set up in Pasteur pipettes by layering 0.5 ml of a suspension containing 1×10^8 sperm ml⁻¹ on to the top of 0.9 ml of 25% BSA (ref. 2). Continuous gradients consisting of 0.3 ml of 10%, 15% and 25% BSA as described by Ericsson *et al.*² were layered on top of each other in Pasteur pipettes and then 0.5 ml of the sperm suspension was carefully layered on top. The columns were left at room temperature for 1 h when the top layer (Tyrode's plus sperm) was removed by pipette. In the continuous gradients the 10% BSA layer was pipetted off after a further 30 min and the final layers after another 30 min. In the single gradients the top layer and the final layer were removed after 1 h. After removal, each layer was washed four times in Tyrode's solution and fixed in methanol: acetic acid (3:1). The fixed suspensions were dropped on to slides and air dried before staining in an aqueous solution of quinacrine dihydrochloride for three minutes. All slides were coded, randomised and scored blind for the presence of a Y body in 100 or 200 sperm from each of two slides made from each layer of each gradient. The observations were made using a Leitz Ortholux microscope fitted with the appropriate fluorescence attachments⁶.

Our results show that the percentage of Y-bearing sperm in the final isolates does not deviate significantly from that of the controls (Table 2) and thus contradict those of Ericsson *et al.*². They do, however, demonstrate that isolation seems to be dependent on sperm motility and that as Ericsson *et al.*²

describe, the non-motile sperm are initially retained in the upper less dense layers (Table 1).

It is well known that spermatozoa in a semen sample from normal fertile human males, in contrast to most other mammals, show considerable morphological heterogeneity⁷ and include a significant proportion of immature cells. The percentage of so-called 'normal' forms (defined largely on shape and structure of head and tail) is, in our laboratory, very often below 50% and rarely above 70%. As Ericsson *et al.*² have noted, selection for motility also results in increasing the proportion of morphologically normal sperm. Our own data (Table 1) clearly support and confirm this conclusion (compare with Fig. 1b and c). With this technique, however, the yield of normal motile sperm is low (between 1% and 5% of the original sperm input) and, although dependent on the quality of the initial sample, could doubtless be increased with improved methods.

There is no doubt, therefore, that fractionation of sperm samples using BSA gradients provides a method for selecting morphologically normal sperm of high motility so the technique might prove useful in the treatment of male subfertility. A series of ejaculates could be treated by the BSA method and the isolates stored in liquid nitrogen until a sufficient sperm concentration is achieved.

Finally we emphasise that our results do not support the contention that there is a differential motility between X- and Y-bearing spermatozoa that can be exploited to separate these cell types using the methods described by Ericsson *et al.*².

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A. ROSS
JACQUELINE A. ROBINSON
H. J. EVANS

MRC Clinical and Population Cytogenetics Unit,
Western General Hospital,
Edinburgh EH4 2XU, UK

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Sex and age differences in human platelet aggregation

MALES stand at risk in many pathological states¹, especially cardiovascular disease, in which there is a statistically significant higher mortality in men than women². We have described³ a sex difference in platelet reactivity in rats and guinea pigs where platelets from males were significantly more sensitive to aggregating stimuli than those from females. Increased platelet responsiveness has been detected in humans,

in thromboembolic disease⁴ and after acute myocardial infarction⁵. Indeed, the principal *post mortem* finding in subjects who died suddenly is occlusion in the pulmonary circulation due to platelet aggregation⁶. We have suggested³ that platelet sensitivity is mediated by the androgenic steroids and that androgens contribute to the incidence of thrombotic disease.

In contrast to the situation with synthetic contraceptive steroids⁷, few variations have been observed in the response of human platelets to endogenous sex hormones, although adenosine diphosphate (ADP)-induced aggregation in women was increased during ovulation and in pregnancy⁹. Zahavi *et al.*⁸ reported that platelets from normal women on the second day of menstruation were more responsive to aggregation than those from normal men. Platelet adhesiveness in the presence of an encephalitogenic factor was also greater in women than men¹⁰, although this was not observed in another study where platelet adhesiveness was estimated directly¹¹.

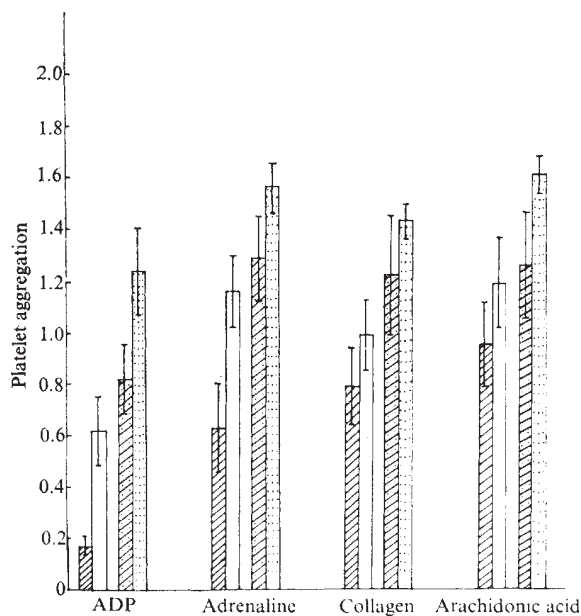


Fig. 1 Effect of sex and age on human platelet aggregation. PRP was prepared from the blood of human male, mean age 23.2 (hatched symbol), mean age 42.4 (hatched/dotted symbol) and female donors, mean age 25.2 (open symbol), mean age 45.9 (dotted symbol), at the same time of day²¹. The platelet count was adjusted to $\sim 200,000 \text{ mm}^{-3}$ with autologous platelet-poor plasma (PPP). The light transmission through PRP and PPP was taken as 0% and 100% aggregation respectively. The extent of maximum aggregation (T_{max} ¹⁷, in light transmission units) was recorded following addition of an aggregating agent to 1 ml of PRP (37°C, 900 R.P.M.) in a siliconised tube. The response to ADP (Equine muscle, Sigma; $1 \mu\text{g ml}^{-1}$), adrenaline (Parke Davis; $10 \mu\text{M}$), collagen (50 μl of suspension)²² and arachidonic acid²³ (1 mM) in 35 estimations ($\pm$ s.e.m.) is indicated.

The mortality rate from cardiovascular disease increases exponentially with age in both men and women, although it is always higher in men and reaches a maximum in the fifth decade of life¹². We have now found that platelet sensitivity to aggregant stimuli similarly increases with age, especially in men, and that donors classified as high mortality risk (on an age basis) have enhanced platelet responsiveness when compared with younger donors of lower risk.

Blood samples were drawn from the cubital vein of resting human volunteers into 3.8% (w/v) trisodium citrate (1 part anticoagulant to 9 parts blood). Donors were screened for recent history of medication¹³, cigarette smoking¹⁴ and no samples were taken within 3 h of a meal¹⁵. Platelet-rich plasma (PRP) was prepared as described previously¹⁶ and maximum aggregation (Fig. 1) was assessed routinely 1 h after sampling, in an aggregometer (Payton Associates, New York). This

parameter is linearly proportional to platelet count and aggregating agent concentration¹⁷ and was observed here to give good reproducibility between repeated tests on the same donor. Primary aggregation, monophasic with pronounced disaggregation in response to ADP ($0.5 \mu\text{g ml}^{-1}$) and secondary aggregation, monophasic exponential or biphasic with minimal disaggregation in response to ADP ($5 \mu\text{g ml}^{-1}$), was demonstrated in each sample before use.

In one study, 19 healthy men, 21–25 yr old (mean 23.2) were compared with 19 healthy women, 21–26 yr old (mean 25.2). In a second study, 17 normal men, 38–47 yr old (mean 42.4) were compared with 16 premenopausal women, 36–50 yr old (mean 45.9). Samples from women were taken at random with respect to the menstrual cycle⁸, but not during menstruation. In a third study, postmenopausal women, 56–58 yr old (mean 57.0) were compared with normal men, 55–62 yr old (mean 58.5). These postmenopausal women were not on oestrogen supportive therapy. Mean values for the platelet response to various aggregating agents in these groups were compared, and the appropriate Student's *t* statistics calculated.

The primary aggregation (reversible) induced by ADP in platelets of young healthy women (mean age 25.2) was significantly ($P < 0.001$) increased over the values obtained in males (mean age 23.2) (Fig. 1). This female platelet sensitivity apparently depended on the degree of aggregating stimulus, and was 3.7 times greater than male sensitivity in response to ADP ($1 \mu\text{g ml}^{-1}$) and 2.0 times greater in response to ADP ($2 \mu\text{g ml}^{-1}$). These data are in accord with the report⁸ that female platelets were more responsive than male platelets to ADP ($0.6 \mu\text{g ml}^{-1}$ and $6.0 \mu\text{g ml}^{-1}$). They are, however, in contrast to our observations in the rat and guinea pig, where male platelet sensitivity was significantly greater than female sensitivity at concentrations of ADP from 0.1 to $10 \mu\text{g ml}^{-1}$. Primary and secondary (irreversible) aggregation induced by adrenaline was also significantly greater (1.8 times, $P < 0.01$) in human females than in males (Fig. 1). No significant difference was observed in secondary aggregation of these platelets induced by either collagen or arachidonic acid.

In the high risk age group (mean 45.7), platelet aggregation in response to primary and secondary aggregating stimuli was significantly ($P < 0.005$) enhanced in both males and females, when compared with the low risk group (mean 24.2). Female platelets were again more responsive (1.25 times) to ADP ($1 \mu\text{g ml}^{-1}$) than male platelets ($P < 0.05$) (Fig. 1). This female/male ratio of sensitivity was, however, significantly less than that in the low risk age group. Indeed, there was no significant sex difference in response to ADP ($2 \mu\text{g ml}^{-1}$) in the older group.

The change in sensitivity ratio was reflective of a significantly ($P < 0.01$) greater increase in male platelet responsiveness in the high risk group. For example, platelet aggregability increased 4.8 times in response to ADP ($1 \mu\text{g ml}^{-1}$) in males and only 2.0 times in females. Results were similar with adrenaline-induced aggregation. In the high risk group there was no significant difference ($P > 0.05$) between males and females in their response to adrenaline (Fig. 1). This is in contrast to the female/male ratio of 1.8 determined in the low risk group and represents a twofold increase in male platelet sensitivity.

The marked sex difference in platelet sensitivity observed in both high and low risk groups could be the consequence of an interaction of platelets with sex hormones. We have demonstrated that natural sex hormones may attenuate platelet behaviour *in vitro* and *in vivo*³. To determine the effects of oestrogen withdrawal¹⁸, platelets from premenopausal women (mean age 45.9) were compared with those from postmenopausal women (mean age 57.0). There was no significant difference in platelet responsiveness to either ADP or adrenaline between these groups, suggesting that oestrogens are not responsible for the enhanced platelet sensitivity in females.

In addition, incubation of male or female human PRP with oestradiol ($1 \mu\text{g ml}^{-1}$) or with progesterone ($1 \mu\text{g ml}^{-1}$), under conditions which detect possible platelet-sensitising effects of

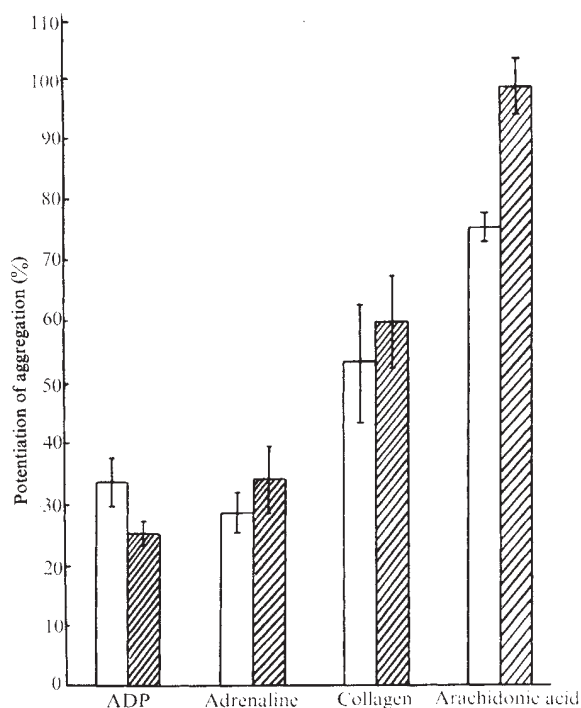


Fig. 2 Effect of testosterone *in vitro* on human platelet aggregation. Human male (open symbol) and female (hatched symbol) PRP (platelet count $\sim 200,000 \text{ mm}^{-3}$) was incubated in the presence or absence of testosterone ($1 \mu\text{g ml}^{-1}$) for 30 min at 22°C . Potentiation (%) of aggregation to ADP ($1 \mu\text{g ml}^{-1}$), adrenaline ($10 \mu\text{M}$), collagen ($30 \mu\text{l}$ of suspension) or arachidonic acid (1 mM) over that in the control (vehicle-treated) plasma is illustrated. The mean of 25 estimations ($\pm \text{s.e.m.}$) is indicated.

the sex hormones (M. J., E. R., and P. W. R. unpublished) resulted in only 10.4 ± 2.4 and $10.9 \pm 0.3\%$ increases in responsiveness to ADP. This was significantly ($P < 0.01$) less than after incubation with androgenic steroids. Testosterone ($1 \mu\text{g ml}^{-1}$) enhanced the aggregating capability in response to ADP by 33.6 ± 3.9 and $24.9 \pm 2.1\%$ in male and female platelets respectively (Fig. 2). It is interesting that testosterone was also more effective than either oestradiol or progesterone in inducing sensitivity to secondary (irreversible) aggregating agents such as adrenaline, collagen and arachidonic acid. Aggregation of both male and female platelets induced by arachidonic acid was very responsive to androgen *in vitro*, exhibiting 75.0 ± 1.9 and $97.0 \pm 4.5\%$ increases in sensitivity respectively (Fig. 2).

Thus we have demonstrated that (a) there is a significant sex and age difference in human platelet sensitivity to aggregating stimuli; (b) female platelets are more responsive than the corresponding male platelets; (c) platelet responsiveness in both sexes increases with age, male sensitivity increasing at a greater rate than female sensitivity; and (d) cardiovascular disease high risk age groups exhibit enhanced platelet responsiveness when compared with low risk groups.

The sex difference in platelet sensitivity may be intrinsic to the platelet. Our evidence indicates, however, that natural oestrogenic and progestogenic steroids have minimal effects on platelets both *in vivo* and *in vitro*, although androgenic steroids were significantly active *in vitro*. Platelet sensitivity may equally be determined by interaction with various clotting factors, fibrinogen, plasminogen and fibrinolytic inhibitors, the levels of which are sex hormone-dependent¹⁹.

Disaggregation⁸ and adhesiveness¹¹ of platelets is apparently not related to the age of the donor. Male platelet responsiveness, however, increased significantly with age and concurrent with other androgen-related risk factors such as pressor sensitivity²⁰, blood viscosity¹ and serum cholesterol, phospholipid and tri-

glyceride levels¹² may contribute significantly to the incidence of arterial disease.

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MALCOLM JOHNSON
ESTELLE RAMEY
PETER W. RAMWELL

Department of Physiology and Biophysics,
Georgetown University Medical Center,
Washington DC 20007

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Endometrial cell calcium and oestrogen action

ADMINISTRATION of oestrogen to an ovariectomised or immature rat promotes a rapid increase in the content of water and univalent electrolytes in uterus and vagina^{1,2}. Although alterations in the uptake and retention of calcium and other divalent cations by several hormone-responsive tissues have been reported following long term administration of oestrogen *in vivo*^{3,4} and during the oestrous cycle⁵, no information is available on the cellular exchange, if any, of calcium shortly after administration of oestrogen in sensitive targets. In view of evidence suggesting a critical role for calcium in the initiation and/or regulation of cell growth and metabolism^{6,7}, consideration of the potential involvement of this cation in the uterine response to oestrogen is important to analysis of steroid hormone action at the cellular level. Using endometrial cell suspensions isolated from the uteri of ovariectomised rats⁸, we have now shown that physiological levels of oestradiol- 17β influence rates of cellular calcium exchange as early as 2.5 min after *in vitro* addition of hormone.

Endometrial cells were isolated and maintained as before⁸ and suspended in Ringer solution composed of 136.9 mM NaCl, 2.7 mM KCl, 0.7 mM CaCl_2 , 0.6 mM MgCl_2 , and buffered at pH 7.4 with 8.1 mM $\text{Na}_2\text{HPO}_4/1.5 \text{ mM KH}_2\text{PO}_4$, in the presence of 1 mM Na pyruvate. Calcium uptake by the isolated endometrial cells was determined from accumulation of $^{45}\text{Ca}^{2+}$ (New England Nuclear Corp.). A 40-ml cell suspension ($\sim 10^6$ cells per ml) in a 50-ml plastic Erlenmeyer flask was placed over a submersible magnetic stirrer in a water bath at 37°C and gassed with O_2 . After a brief equilibration period, 50 μCi of $^{45}\text{CaCl}_2$ was added (zero time) and incubation continued. Samples of medium were taken at selected intervals after addition of the isotope. For each time interval, two samples (1.5 ml each)

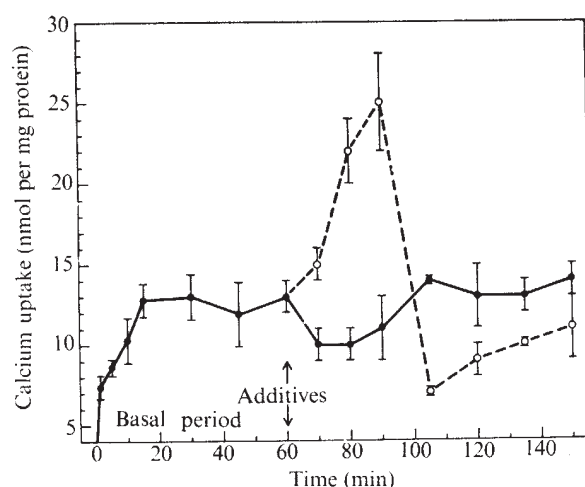


Fig. 1 Calcium uptake of isolated endometrial cells is expressed as (d.p.m. per mg cell protein)/(d.p.m. per nmol medium calcium). After a 60 min basal period, oestradiol-17 β (○), to a final concentration of 1×10^{-8} M, or its vehicle, ethanol (●), to a final concentration of 0.02%, were added *in vitro* to experimental and control suspensions, respectively. The incubations were then continued for 90 min. Each point (mean $\pm$ s.e.m.) represents data from three to six independent experiments.

were removed and transferred into two plastic centrifuge tubes containing 11 ml ice-cold, isotonic choline chloride, buffered at pH 7.4 with Tris. The tubes were immediately centrifuged (at 4° C in a Sorvall SC2B centrifuge) at 600g for 1 min. The supernatant was decanted, and the tubes were inverted to drain overnight. Double-distilled, deionised water (4 ml) was added to the cell sediment which was homogenised with a high-intensity ultrasonic probe. Aliquots of sonicate were taken for determination of cell protein⁹ and ⁴⁵Ca by liquid scintillation counting. Oestradiol-17 β , where indicated, was added to the medium to a final concentration of 1×10^{-8} M 60 min after zero time; the control flask received vehicle alone.

Efflux of ⁴⁵Ca was determined with cells preloaded with isotope by the addition of ⁴⁵CaCl₂ (final concentration of 50 μ Ci ml⁻¹), followed by 60 min of incubation. The suspension was washed by centrifugation twice with buffer for 1-min periods at 600g. The final sediment was resuspended in 6 ml of ⁴⁵Ca-free Ringer solution and one-half distributed into each of two stop-flow chambers. The chambers, made from polycarbonate, were mounted on a filter funnel equipped with a stopcock which could be opened for rapid filtration of the suspension medium through a 0.45- μ m Millipore filter. The top of the chamber was sealed with a rubber serum cap through which O₂ and wash solutions could be introduced by means of syringe needles. This assembly was immersed immediately above a submersible stirring magnet in a water bath at 37° C. A 1 mm bar magnet stirred the suspension continuously.

Isotope desaturation began with the addition of cell suspensions to the chambers. The medium was replaced at selected times, when cells were washed with 2 $\times$ 3 ml 'cold' Ringer solution with the stopcock open. The stopcock was then closed and 3 ml of fresh medium was delivered to resume the desaturation. Washing was completed within 30 s. Aliquots of the spent media were taken for liquid scintillation counting of ⁴⁵Ca. After desaturation, cells were collected and sonicated as above. Aliquots of sonicate were taken for determination of total cell ⁴⁵Ca and protein as above.

Figure 1 shows that from zero time to 60 min, accumulation of calcium in the cells in the absence of test agents was exponential, similar to that described for other mammalian cells¹⁰. Addition of oestradiol increased Ca²⁺ uptake to 153% of control levels in 10 min ($P < 0.05$) with a peak

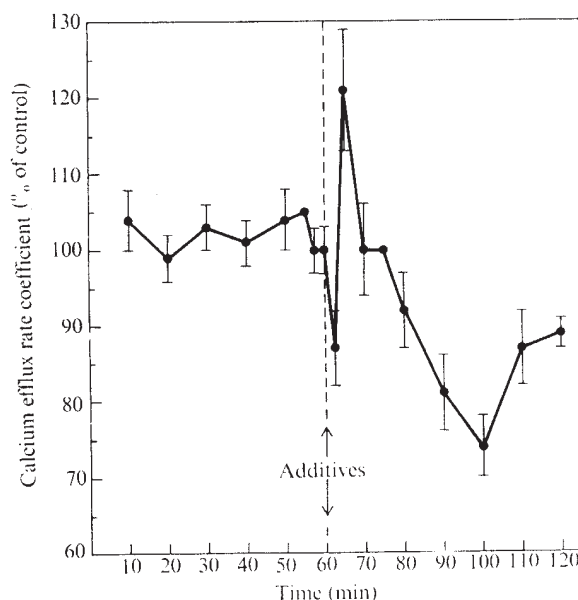
at 230% of controls ($P < 0.01$) within 30 min. Uptake declined to 50% of control values ($P < 0.01$) by 45 min and then gradually rose towards the baseline.

To test whether oestrogen increased net calcium accumulation in part by inhibiting efflux, we measured calcium efflux rate coefficients in the presence and absence of oestradiol-17 β , added *in vitro*. Cells were preloaded with isotope for 60 min and then distributed equally into an experimental and a control group. Figure 2 shows that during the first hour of desaturation, represented by the basal period, efflux occurred at essentially the same rate in both samples. After addition of oestradiol at 60 min, however, ⁴⁵Ca²⁺ efflux began to oscillate both above and below control levels. It declined to 87% of control after 2.5 min ($P < 0.05$), rose to 121% of control within 5 min ($P < 0.05$) and then declined to its lowest ebb, at 74% of control ($P < 0.001$), 40 min after addition of hormone. Thereafter, it remained depressed ($P < 0.05$) to the termination of desaturation measurements at 120 min.

The relationship of these early changes in calcium flux to later metabolic effects of oestradiol was considered in further experiments. We have shown previously that cortisol can block the stimulatory effect of oestradiol on glucose metabolism of isolated endometrial cells⁸. The action of 3×10^{-6} M cortisol hemisuccinate (Solu-Cortef, Upjohn) and its interaction with 1×10^{-8} M oestradiol in affecting ⁴⁵Ca²⁺ accumulation were evaluated from measurements of calcium uptake taken for 30 min after addition of steroids to isolated cells; ethanol to a final concentration of 0.02% was added to suspensions of control cells. Cortisol alone did not affect Ca²⁺ uptake, for in its presence uptake was 95% of controls. Addition of cortisol immediately before exposure to oestradiol, however, abolished the expected increase of calcium influx within 30 min (94% of control). These results parallel those obtained for intestinal calcium transport which indicate that glucocorticoids antagonise vitamin D-induced absorption of calcium¹¹.

Our experiments demonstrate that physiological concentrations of oestradiol-17 β act *in vitro* to produce oscillations

Fig. 2 The calcium efflux rate coefficient of isolated endometrial cells was calculated according to Isaacson and Sandow¹⁵. With the rate coefficient of the control group being taken as 100%, the changes in the oestradiol-treated group at each point were expressed as percentage of control. Additives were introduced *in vitro* at the concentrations indicated in Fig. 1 after a 60-min basal period. Each point (mean $\pm$ s.e.m.) represents data from three to seven independent experiments; points without s.e.m., however, are taken from the results of two experiments.



in the net calcium content of isolated endometrial cells by influences on rates of influx and efflux. Similar variations in the cellular content of calcium have been considered important in the action of other hormones¹². Other evidence also indicates that increments in cellular calcium accumulation precede and/or trigger cell division and growth^{6,7}, which is the end-point of oestrogen action in endometrium. Strict control of intracellular calcium seems to be achieved by regulation of fluxes across the plasma membrane and between the cytoplasm and subcellular organelles, such as mitochondria¹¹. It has also been reported that diethylstilboestrol blocks energy-dependent Ca^{2+} uptake by mitochondria isolated from the myometrium¹³. Collectively, these oestrogenic effects on calcium exchange must lead to marked changes in cytoplasmic Ca^{2+} activity, binding and redistribution at intracellular sites. The characterisation of calcium exchange in resting and oestrogen-stimulated endometrial cells, therefore, has significant bearing on the processes involved in metabolic and mitogenic responses to the hormone¹⁴.

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RICHARD J. PIETRAS
CLARA M. SZEGO

Departments of Physiology and Biology and
Molecular Biology Institute,
University of California, Los Angeles,
California 90024

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Two biological activities regulating cell proliferation found in cultures of peritoneal exudate cells

We have found two distinct biological activities in cultures of peritoneal exudate cells rich in macrophages. One consists of an inhibitory effect on cell proliferation, described previously, caused by a low molecular weight product¹; the other is a stimulatory effect brought about by a non-dialysable material. Both activities were found in the same culture fluid; and different kinds of cells exhibited different sensitivities to them. Our results offer a possible explanation for the discrepancies in the literature concerning the different effects of macrophages or their products on cells^{1–7}.

We cultured the peritoneal exudate from A/St or C57BL/6 mice injected 3 d previously, intraperitoneally, with 1.5 ml of a 10% (v/v) solution of proteose peptone (Difco). The cells were plated at a density of 10^7 or 2×10^6 cells per ml on 35 mm plastic dishes (Falcon) in RPMI-1640 culture medium supplemented with 5% foetal calf serum. After 24 h, the medium was collected; the cell monolayer

was washed, fresh medium was added, and the culture continued for 48 h. The activities of the culture fluids obtained during the first 24 h or from the 24–72-h period were tested. Most cells forming the exudate were typical macrophages as judged morphologically; during the first 24 h, 5–10% lymphocytes were present with the macrophages, but most were eliminated by the washing at 24 h. We tested the incorporation of ^3H -thymidine by EL-4 leukaemia cells, P815 mastocytomas cells, thymocytes and spleen cells cultured in the presence of various amounts of fluids from macrophage cultures. Results have been consistent for more than twenty different experiments. The main results shown in Figs 1–3 were obtained with a fluid from a culture of 10^7 cells per ml.

Incorporation of ^3H -thymidine into EL-4 leukaemia cells cultured for 8 h in the peritoneal cell supernatant was inhibited markedly (Fig. 1). This inhibition correlated with that of cell mitotic activity and with the increase of cell density with time of culture¹. The culture fluid, if dialysed extensively against normal culture medium, lost all inhibitory activity. EL-4 cells cultured in dialysed culture supernatants incorporated the same amount of thymidine as cells cultured in normal medium. Another cell line, the mouse mastocytoma P815 used extensively in current *in vitro* studies of cell-mediated immunity, behaved as the EL-4 line, and its incorporation was inhibited markedly by the supernatants (data not shown).

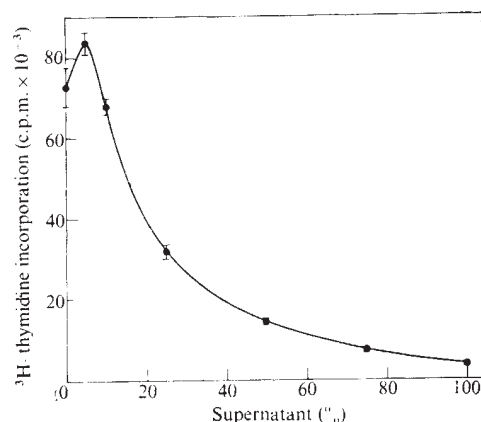


Fig. 1 Incorporation of ^3H -thymidine by EL-4 leukaemia cells cultured in the presence of different amounts of culture fluids from peritoneal exudate cells. Cells were cultured at a density of 5×10^5 per ml in plastic tubes for 8 h, the last 4 h in the presence of ^3H -thymidine (New England Nuclear Corp., 2 Ci mmol⁻¹). Each result is the mean of three culture tubes. Brackets refer to standard error of the mean. In this experiment, the EL-4 cells (a tumour line from C57BL/6 mice) were cultured in the presence of fluids from A/St mice. Identical results have been obtained if the cells are cultured in fluids from C57BL/6 peritoneal cells.

We examined for the amounts of inhibitor extracted by freeze-thawing the peritoneal exudate cells or released into the medium after culture for 24 h. The amounts of material (v/v) required to reduce by 50% the incorporation of ^3H -thymidine by EL-4 cells were determined. The amounts were: 63% from cells lysed immediately after plating; 83% from cells lysed 24 h after plating, and 22% from the fluids after culture for 24 h. Thus, the inhibitory activity found in culture fluids was generated during the culture and did not result from lysed or dead cells. The chemical nature of this material is not known. In our previous experiment, we indicated that the material was resistant to tryptic digestion, to phosphodiesterase treatment, and to boiling and freeze-thawing¹.

The spontaneous incorporation of ^3H -thymidine into spleen cells cultured with the untreated supernatant was inhibited slightly. When these cells were cultured with

dialysed supernatants, c.p.m. were the same as controls in the range of doses used, 10–50% (v/v). (In other experiments, a 75% concentration of the dialysed peritoneal cell supernatant increased incorporation of ^3H -thymidine by about 90%.) If spleen cells were cultured in the presence of phytohaemagglutinin (PHA) for 3 d, there was an expected marked increase in incorporation (^3H -thymidine was present during the last 12 h of culture) (Fig. 2). The culture conditions used here—3-d culture, 1640 medium with 5% foetal calf serum, 10^6 lymphocytes in 12×75 -mm plastic tubes, 12-h pulse of ^3H -thymidine, and 100 μl of 1:100 dilution of Difco PHA-P—were selected to produce optimal response. The response to PHA was reduced markedly in the presence of peritoneal cell culture supernatant in agreement with our findings¹. The dialysed culture supernatant, however, not only lost its inhibitory property but increased the response of the spleen cells to PHA. This experiment, therefore, revealed two activities differentiated on the basis of size, influencing the multiplication of spleen lymphocytes.

Of particular interest are the results with thymocytes. The conditions for thymocyte culture were identical to those used for spleen cells, except for the cell density, which was 5×10^6 cells per ml. First, the untreated supernatants increased slightly the spontaneous incorporation of thymidine into thymic cells; the dialysed fluid, however, had markedly more activity (Fig. 3). Clearly, the fluids contained a principle that stimulated ^3H -thymidine incorporation and which was partially masked by a low molecular weight inhibitor. Thymocytes responded poorly to PHA as expected. For example, the spontaneous c.p.m. of thymocytes after culture for 3 d was 678 (mean of triplicate culture; s.e.m. ± 115); PHA for 3 d increased this only to 1,337

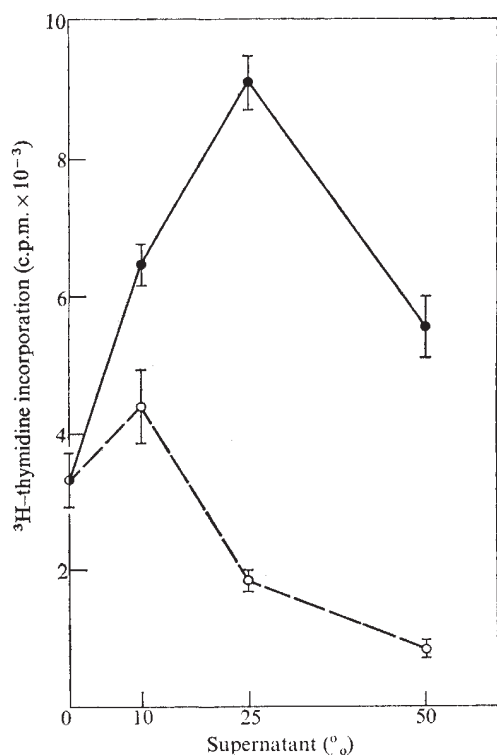


Fig. 2 Spleen cells from A/St mice were cultured in the presence of various amounts of the supernatants and PHA. Time of culture was 72 h, the last 12 h in the presence of ^3H -thymidine. Cultured fluids were dialysed against ten times the volume of normal medium for 72 h, changing the dialysate on four occasions. ●, Dialysed supernatant; ○, untreated supernatant.

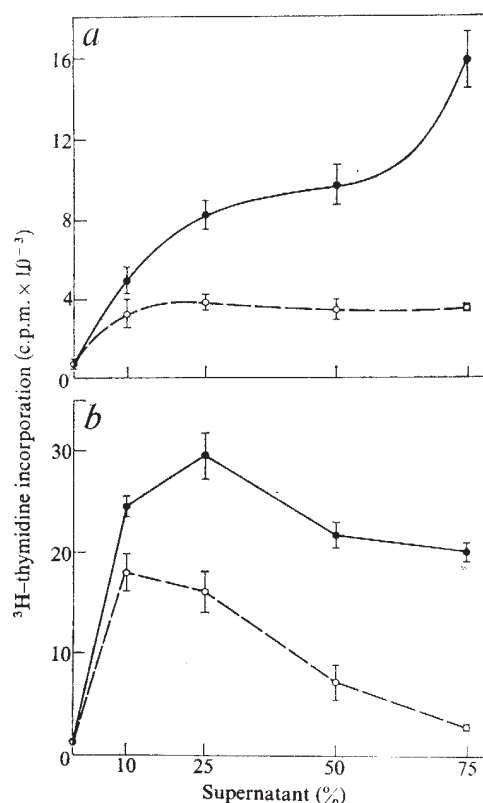


Fig. 3 Response of thymocytes to supernatants of peritoneal exudate cells. For explanation see text. a, No PHA; b, with PHA. Symbols are as in Fig. 2.

(± 109). In agreement with the data of Gery *et al.*², the untreated culture supernatant increased the PHA response of thymocytes up to $18,253 \pm 1,962$, at 10% (v/v) concentration. This culture supernatant inhibited at high concentrations (more than 10%). The dialysed supernatant, on the other hand, was much more potent and showed, at the most, a small decline at high concentrations.

In other experiments we have dissociated the inhibitory and stimulatory activities by varying the density of the peritoneal exudate cells. All results described before were obtained with culture fluids obtained from 10^7 cells per ml plated in 35-mm plastic dishes. A cell density of 2×10^6 per ml produced supernatants in which inhibitory activity was decreased in proportion to the reduction in cell density but in which stimulatory activity was maintained at about the same intensity.

An inhibitor or a stimulatory factor for cell proliferation released from peritoneal exudate cells explains some of the divergent results previously published, but raises important points concerning their chemical nature and biological significance. All evidence points to the macrophage as the source of both materials since peritoneal exudate cells devoid of lymphocytes released the same amount of activity (ref. 1 and unpublished observations). The relationship between the state of maturation and/or activation of the macrophages and the two factors, however, are not clear. It is possible that in a given situation inhibition or stimulation could result from such conditions as number of macrophages, anatomical relationship between macrophages and the target cell, the state of the macrophage and the nature of the target cell in question. The two factors must now be considered with regards to the well known role of macrophages in immune induction⁸ and in cellular type of immunities⁹.

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JESUS CALDERON
EMIL R. UNANUE

Department of Pathology,
Harvard Medical School,
Boston, Massachusetts 02115

Received October 7; revised November 18, 1974.

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Chromosomal variation and the establishment of somatic cell lines *in vitro*

WHEN somatic cells from a number of species are explanted *in vitro*, they may either completely fail to grow, or they may continue to divide for a limited number of generations, at a progressively lower rate and with decreasing plating efficiency. They will eventually die unless, from this "crisis", a new population emerges with altered properties (see for example ref. 1). This new population, the established line, is usually capable of existing indefinitely and its growth rate and plating efficiency progressively increase until they stabilise at a level characteristic of each individual line.

The chromosomal constitution of these established lines is virtually always different from that of the parental primary cells and it is still a matter of debate whether these karyotype changes precede or follow establishment.

A parasexual cycle of polyploidisation-segregation has been found in human fibroblasts before crisis² and together with the fact that senescent human fibroblasts can be rescued by cell fusion³—which is normally followed by chromosome segregation⁴—suggests that polyploidisation may be important for establishment, perhaps as a source of variability. As the number of centromeres is subject to certain restrictions⁵ and multipolar mitoses are frequent⁶ the polyploid fraction would be unstable and would tend to generate a whole range of new types, often pseudo or quasi-diploid. Such variations in gene balance would give greater opportunities for generating types capable of existing indefinitely. Therefore polyploidisation, no matter how induced, could promote the growth of primary cells explanted *in vitro*. To test this hypothesis, we used various treatments which ultimately induce polyploidisation to determine whether the treated cell populations showed a better adaptation to *in vitro* conditions.

Whole mouse embryo cells, 4 d after explantation, were treated with β -propiolactone inactivated Sendai virus to promote cell fusion and then seeded in agar. Four colonies developed from a total of 5×10^6 Sendai-treated cells whereas no colonies developed from 5×10^7 untreated control cells. This growth could not be caused by growth-promoting factors present in the virus suspension since the virus had no effect on sparse cells in monolayers. Moreover, cell fusion rather than simple cell aggregation seems to be responsible because the colonies grown in agar, when isolated, propagated in monolayer and replated in agar, showed a plating efficiency of approximately 10^{-4} .

Chick and hamster cells were also treated with polyploidising agents such as griseofulvin, cytochalasin B or inactivated Sendai virus. Whole embryo primary cells, 4 d after explantation, were trypsinised and either treated with inactivated Sendai virus (200 haemagglutinating units per ml for 30 min) and then plated at 1.5×10^4 cells cm^{-2} , or plated

at the same concentration and treated the next day with griseofulvin ($50 \mu\text{g ml}^{-1}$) or cytochalasin B (1 and $5 \mu\text{g ml}^{-1}$). (In the case of chick cells, the plating concentration was 10^4 cm^{-2} .) Ten hours later the drug was removed. After 3 d all dishes were trypsinised and the cells plated at 500 cm^{-2} (1,000 in the case of chick cells).

At this cell density the control cells did not grow whereas colonies developed in the other dishes. Some of these colonies were isolated and propagated in monolayer. They gave rise to established lines with a success rate greater than 0.3 in the case of mouse and 0.12 in the case of hamster. With chick fibroblasts no permanent lines were obtained but the proliferation time was extended (85 d in griseofulvin-treated cells compared with 40 d for the control).

The growth-promoting effect can also be measured in terms of mitotic index (Table 1). A few days after treatment the mitotic index increased but there was a concurrent decrease in the polyploid fraction. This indicated that the proliferating fraction might not be represented by polyploid cells but by some of their segregation products.

Table 1 Mitotic index and frequency of polyploids

Time (d)	Control		Cytochalasin treated	
	Mitoses (%)	Polyploid (%)	Mitoses (%)	Polyploid (%)
1	8.3	2.3	4.0	25.0
2	4.7	8.1	3.2	23.2
4	1.8	6.2	1.8	23.2
5	1.9	7.3	4.6	14.7
6	1.6	5.9	5.2	13.0

Mitotic index and frequency of polyploids (cells with more than 60 chromosomes) in whole embryo mouse cells treated with cytochalasin B. Primary mouse cells were trypsinised 4 d after explantation and seeded at 1.5×10^4 cells cm^{-2} . Next day cytochalasin B was added at $1 \mu\text{g ml}^{-1}$ and removed 18 h later (time zero). Each subsequent day coverslips were set up with 2×10^4 cells cm^{-2} . Next day colchicine was added and 3 h later the coverslips were stained.

This possibility was examined with cells from *Mesocricetus auratus* which are generally difficult to establish and tend to be pseudo-diploid and whose chromosomes are easily identified⁹⁻¹¹. We observed a progressive loss of viability *in vitro* with these cells. In six generations the plating efficiency dropped from 0.52 to less than 0.001 and the growth rate, initially 1.5 d^{-1} fell to 0.4 d^{-1} . This only occurred however, if the cell density was kept above 5×10^3 cells cm^{-2} ; at lower cell densities, untreated cells did not grow, whereas cells treated with any one of the polyploidising agents gave rise to colonies, 13 of which were isolated and developed into established lines. But when we seeded treated or untreated cells at densities ranging from 5×10^3 to 5×10^4 cells cm^{-2} and subsequently passaged the confluent dish that had the smallest inoculum, we succeeded in establishing lines from these mass cultures. The properties of these lines, 7 months after continuous cultivation, were indistinguishable from those of the 13 lines obtained after polyploidisation in respect of plating efficiency, which varied between 0.05 and 0.40, and growth rates, which were between 0.8 and 1.5 d^{-1} . When tested 5 months after explantation, two treated and one control line gave palpable tumours in less than 1 month when 3-month-old animals were injected subcutaneously with no fewer than 10^5 cells.

The chromosomal constitution of all these lines was followed from the first passage after explantation for 7 months. The number of polyploid mitoses varied initially from zero (primaries, untreated) to 40% (cells treated with $5 \mu\text{g ml}^{-1}$ cytochalasin B). The difference between treated and untreated cultures decreased however, and by the third week clear differences were no longer observed. In most of the 130 preparations examined, the percentage of polyploids was between 5 and 25. Quasi-diploid cells were in the majority but a stem line could not be identified¹².

When chromosomes were analysed for their banding pattern no real diploid metaphases could be found after the third week in all cases. The most common departures from normality were trisomies and monosomies and the number of translocations and other detectable structural aberrations, at up to five months, was usually no more than 2 or 3 per metaphase.

The conclusion we can draw from this set of experiments is that the karyotypal heterogeneity, known to be common in established lines¹³ begins soon after explantation, much sooner than suggested by earlier studies^{1,8,10}. Polyploidisation and the successive appearance of pseudo and quasi-diploid cells plays an important role in the evolution leading to establishment. This type of karyotype evolution may indeed be a rather general phenomenon since similar steps have been described *in vivo* during liver regeneration and in the bone marrow of adult and ageing hamsters^{12,14,15}.

M. TERZI
T. S. C. HAWKINS

Laboratorio di Mutagenesi e Differenziamento (CNR)

Via Cisanello, 147-56100 Pisa, Italy

and

Imperial Cancer Research Fund,

London WC2A 3PX, UK

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H₁ and H₂ receptors in the histamine-induced accumulation of cyclic AMP in guinea pig brain slices

EVIDENCE has accumulated suggesting that several transmitters, as well as hormonal substances, mediate their actions on effector cells by an increase in the intracellular level of adenosine cyclic 3',5'-monophosphate (cyclic AMP)¹.

Like other biogenic amines, histamine is able to stimulate the cyclic AMP-generating system in brain slices of different animal species². Moreover, this action of histamine on brain cells, like other cerebral actions (either electrophysiological³ or behavioural⁴), seems to be mediated by means of an interaction with specific receptors, as it is not blocked by adrenergic antagonists but prevented by classical antihistamines⁵. In peripheral tissues two classes of histamine receptors seem to be present as evidenced by specific antagonists or agonists. Ash and Schild⁶ have termed H₁ those blocked by classical antihistamines and H₂ the others, responsible, for example, for the gastric secretory effect of the amine. Since the discovery of specific antagonists and agonists of these H₂-receptors⁷, the existence of an H₂-mediated stimulation of adenylyl cyclase has been reported, as in fundic gastric mucosa of guinea pig⁸. We report the presence in brain of both classes of receptors involved in the histamine-induced accumulation of cyclic AMP.

Cyclic AMP accumulation was studied in an *in vitro* model, derived from that described⁹. Male Hartley guinea pigs (300 g) were killed by decapitation, their brain removed and slices of cerebral cortical gray matter (250 µm thick)

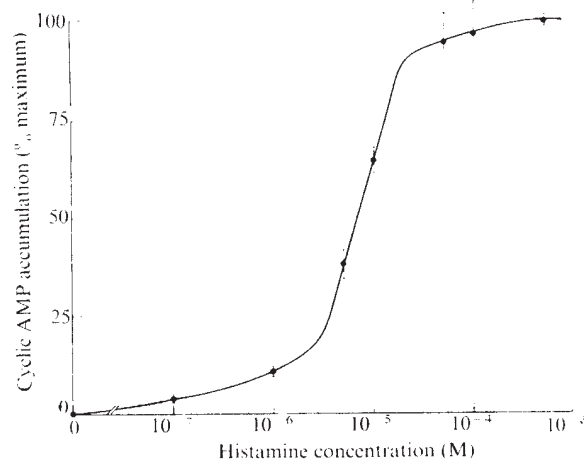


Fig. 1 Effect of histamine on cyclic AMP accumulation in guinea pig cortical slices. In the absence of added histamine 5.3 ± 0.3 pmol per mg protein of cyclic AMP were accumulated. The percentage of maximal accumulation of cyclic AMP, which represents $381 \pm 6\%$ of the basal level, is plotted as a function of histamine concentration. Mean $\pm$ s.e.m. of five samples.

were rapidly prepared with a McIlwain tissue slicer. The slices were preincubated at 37° C for 30 min in the presence of 4 µM adenine in a Krebs-Ringer bicarbonate medium (about 50 ml per g of slices), under a constant stream of a mixture of O₂:CO₂ (95:5) in a Dubnoff metabolic shaker. The slices were then pooled, washed twice with fresh medium and distributed in incubation tubes, about 7.5 mg of tissue per tube. After the addition of test substances, the tubes were gassed and capped; the incubations were continued at 37° C and terminated by homogenisation of the slices with a sonicator. Homogenates were heated at 95° C for 8 min; after centrifugation, the amount of cyclic AMP formed in the slices was measured in 10 µl aliquots of the supernatant by the protein kinase binding method⁹. All test agents did not compete for the binding of cyclic AMP and the specificity of the assay was tested by incubation of selected samples with purified beef heart phosphodiesterase. Proteins were determined by the method of Lowry *et al.*¹⁰ with bovine serum albumin as standard.

Preliminary experiments indicated that the level of cyclic AMP in control slices was constant within at least 15 min of incubation, that the accumulation of cyclic AMP induced by 100 µM histamine reached its maximal value after 10 min and that it remained at this level until 15 min. In further experiments, incubations were therefore continued for 10 min after the addition of the different test substances. Figure 1 shows the increased accumulation of cyclic AMP in cortical slices as a function of histamine concentration; the maximal effect was achieved by 50 µM histamine and the half-maximal stimulation was obtained with 7 µM histamine.

Two specific histamine antagonists were used to assess the characteristics of the cerebral receptors involved in the accumulation of cyclic AMP: mepyramine, a classical antihistamine, which antagonises the activation of the H₁-receptors⁶; and metiamide, a new antagonist of the H₂-mediated histamine response¹¹. Figure 2 shows the effect of increasing concentrations of mepyramine or metiamide on the maximal accumulation of cyclic AMP induced by 50 µM histamine. Both these agents were able to block the effect of histamine in a dose-dependent manner, although to a limited extent, that is, about 50%, in each case. The ID₅₀ (concentration of the drug giving 50% of its maximal inhibition) of mepyramine and metiamide were 0.06 µM and 6 µM, respectively, and the maximal antagonism was achieved by 1 µM and 100 µM.

Table 1 Effects of various effectors on cyclic AMP (pmol per mg protein) accumulation in guinea pig cortex slices

Agonist	None	Mepyramine (1 μ M)	Antagonist	Metiamide (100 μ M)	Mepyramine (1 μ M) + metiamide (100 μ M)
None	4.27 $\pm$ 0.20	3.60 $\pm$ 0.40*		4.88 $\pm$ 0.46*	
Histamine (50 μ M)	9.71 $\pm$ 0.56†	6.22 $\pm$ 0.78†		6.44 $\pm$ 0.25†	4.58 $\pm$ 0.26*
4-Methylhistamine (10 μ M)	5.39 $\pm$ 0.46†				
(50 μ M)	6.78 $\pm$ 0.64†				
(100 μ M)	7.59 $\pm$ 0.38†	7.32 $\pm$ 0.31†		4.99 $\pm$ 0.29*	

* Not significantly different from controls.

† Significantly different from controls, $P < 0.05$.Mean $\pm$ s.e.m. of 3–8 experiments.

Moreover, when the two antagonists were added together, the histamine-induced stimulation was completely abolished (Table 1). The effect of 4-methylhistamine, a H_2 -agonist devoid of any significant H_1 -mediated action⁷, was evaluated to further characterise the cerebral histamine receptors. 4-Methylhistamine stimulated the cyclic AMP accumulation in a dose-dependent manner; the response was not affected by mepyramine (1 μ M) but was completely abolished by metiamide (100 μ M) (Table 1).

Our results agree well with previous reports on histamine stimulation of cyclic AMP synthesis in the guinea pig cortex^{2,5,12}. The partial blockade of the response of histamine caused by mepyramine, a classical H_1 -antagonist, and metiamide, a new H_2 -antagonist, clearly suggests the involvement of both H_1 - and H_2 -receptors in the effect of histamine on the cyclic AMP generating system. Chasin *et al.*¹² have found that classical antihistamines were not able to block completely the effect of histamine. Further support for the presence of H_2 -receptors is provided by the fact that 4-methylhistamine, a specific H_2 -agonist, also stimulates cyclic AMP synthesis, an effect blocked by a H_2 -antagonist and not modified by a H_1 -antagonist. Moreover, the additive effects of the two antagonists suggest that the activation of one class of receptor does not affect the activation of the other.

The apparent 'dissociation constant' of histamine for the complex 'receptor-cyclic AMP generating system' is about 7 μ M and does not differ from that reported¹³ for human astrocytoma cells¹³; however, it is far higher than the dissociation constants of histamine for H_1 - and H_2 -receptors determined in tissues containing an homogeneous population

of each receptor (0.09 μ M (ref. 14) and 0.6 μ M (ref. 7), respectively). On the other hand, the ratio of ID_{50} of mepyramine and metiamide is of the same order of magnitude as the ratio of their dissociation constant in peripheral tissues^{6,11}. Therefore, our results suggest that the H_1 - and H_2 -receptors in the brain present common characteristics with those involved in the various actions of histamine in peripheral organs. The fact that we measured the histamine-induced cyclic AMP accumulation in slices, that is, the net result of a complex sequence of interactions, could explain the discrepancies in the dissociation constants for histamine.

In peripheral tissues containing the two classes, H_1 - and H_2 -receptors are often associated with dual effects of histamine, as in the superior cervical ganglion¹⁵. It is tempting, therefore, to speculate that in brain also, the increased intracellular level of cyclic AMP induced by histamine mediates dual effects according to the type of receptor activated by the amine. This could be the case if the different receptors are localised on different classes of brain cells. Consistent with this view is the histamine-induced accumulation of cyclic AMP in human astrocytoma cells¹³, suggesting the presence of histamine receptors on glial cells, whereas the same action on rat cortex synaptosomes¹⁶ suggests the presence of histamine receptors on nerve cells. On the other hand, the possibility that the same cerebral cell possesses the two types of receptors cannot be excluded; in this case the dual responses of the cell to histamine could reflect a localisation on different parts of the membrane and/or the triggering of cellular mechanisms which may occur with different time scales. In other respects, that at least one of the two types of receptors may be localised at cortical synapses is suggested by the recently demonstrated existence of an ascending histaminergic pathway in the medial forebrain bundle projecting into the whole telencephalon^{17,18}.

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MICHEL BAUDRY

MARIE-PASCALE MARTRES

JEAN-CHARLES SCHWARTZ

Unité de Neurobiologie de l'INSERM,

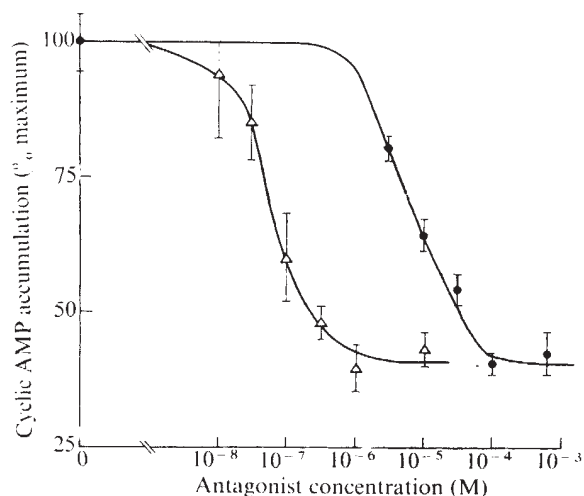
2ter, Rue d'Alésia,

75014 Paris, France

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Fig. 2 Effect of mepyramine (Δ) and metiamide ($\bullet$) on the maximal accumulation of cyclic AMP induced by histamine. The percentage of the cyclic AMP accumulation induced by 50 μ M histamine is plotted as a function of the concentration of each antagonist. The basal level of cyclic AMP in the absence of histamine (4.4 ± 0.4 pmol per mg protein) was not modified by the antagonists at any concentration. Mean $\pm$ s.e.m. of four samples.



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Experimental allergic encephalomyelitis: dissociation of neurological symptoms from lipid alterations in brain

EXPERIMENTAL allergic encephalomyelitis (EAE) is an auto-immune, paralytic disease that can be induced in experimental animals by injection of whole tissue from the central nervous system (CNS), or the purified myelin basic protein (PBP) homogenised in Freund's adjuvant. In some respects, it resembles certain human demyelinating diseases^{1,2}. Preferential changes occur in the metabolism of glycosphingolipids in the CNS³⁻⁶, some of which do not seem to be directly related to the presence of clinical symptoms⁴. In the guinea pig, changes in some of the myelin components seem to depend on the disease-inducing substance used. The cerebroside content of the CNS changes when this animal is sensitised with whole CNS, or with PBP and CNS lipids, but not when PBP alone is used as encephalitogen. The content of sulphatides changes the extent depending on the composition of the encephalitogenic preparation injected⁶. We report here that changes in the level of glycosphingolipids may arise in response to components other than the myelin basic protein and without the simultaneous occurrence of paralytic symptoms.

We have studied the brain lipid content of guinea pigs injected with mixtures of CNS lipids and non-encephalitogenic proteins. The latter preparations were chosen to include basic or non-basic proteins mixed or not with CNS lipids. There are some indications that the susceptibility to different CNS antigens may vary with the strain of animals used and this, in turn, may influence the magnitude of lipid alterations in EAE^{3,5,6}. As mixed bred animals from different sources were used in our studies, controls injected with encephalitogenic preparations were included to compare the lipid alterations found in the EAE animals with those obtained previously⁶.

Forty random bred, adult guinea pigs, of mixed colour and 500-900 g body weight, were randomly distributed in eight

groups and injected intradermally in each hind foot as in Table 1. The amounts of each lipid and PBP injected to animals of groups BP and BPL corresponded to the quantities of these components normally present in the amount of frozen rat spinal cord administered to group CNS. The quantity of PBP used is well in excess of the amount required to produce a high incidence of acute EAE in guinea pigs⁷. Thirteen days after injection the characteristic symptoms were present in all the animals injected with the encephalitogenic preparations (groups CNS, BP and BPL) whereas no neurological effects could be seen in the guinea pigs from groups CF, Ab, Py, AbL and PyL. All of the animals were killed by decapitation 14 days after the injection, the brain quickly removed and its lipid content analysed. Details of the preparation of PBP and CNS lipids and quantitative determinations employed were described previously⁶.

Table 1 shows that the differences in the contents of cerebroside, sulphatides and gangliosides in animals with paralytic symptoms were in general similar to those recently reported for groups CNS, BP and BPL (ref. 6). The changes for gangliosides were again not very significant. We confirmed our previous observations that the injection of PBP (group BP) induced a decrease in the content of sulphatides but not in cerebroside and that the inclusion of lipids in the injection mixture (group BPL) is necessary to induce alterations in the cerebroside levels and to lower significantly the sulphatide content⁶. The animals from groups Ab, Py, AbL and PyL did not differ in physical appearance from the controls (group CF), nor did albumin-injected animals (group Ab) differ significantly in any of the lipids studied. The injection of the basic protein poly-L-lysine (group Py) produced a pattern of alterations closely resembling that found in animals injected with PBP. The importance of lipids in the injection mixture for inducing the changes without the need for encephalitogenic protein and without the concomitant appearance of neurological effects is clearly indicated in groups AbL and PyL. In these groups the changes were very similar to those observed in animals from groups BPL and CNS. The whole mixture of lipids or only particular components could be necessary.

Since the method used for the determination of sulphatide may be altered by other lipids, and values for cerebroside have been obtained by difference⁶, a statistical comparison was made of the total hexose content of the monohexosyl ceramide fractions (cerebroside plus sulphatide). The results obtained

Table 1 Lipid content of the brain in EAE and non-EAE guinea pigs

Animals	Total lipids (mg per g wet weight)	Sulphatides (mg %)	Cerebroside (mg %)	Gangliosides (μ mol sialic acid %)	Total cholesterol (mg %)
Group CF*	81.94 ± 3.53	5.76 ± 0.25	14.86 ± 0.94	2.08 ± 0.13	19.71 ± 0.45
Percentage differences with respect to group CF					
EAE					
Group CNS	1.7	-54.7§	-45.9§	-23.6‡	0.5
Group BP	6.0	-31.3§	-1.3	-20.7‡	0.1
Group BPL	2.6	-50.7§	-52.2§	-18.8‡	4.4
Non-EAE					
Group Ab	2.7	-17.0	4.8	1.9	1.9
Group AbL	6.0	-53.3§	-32.6†	-14.9	-3.6
Group Py	1.9	-42.5§	1.3	-16.3‡	5.6
Group PyL	6.2	-66.3§	-25.4§	-22.1‡	1.1

Each group comprised five animals and the results are mean values $\pm$ s.e.m. Animals were injected intradermally in each hind foot according to the following scheme. Group CF, 0.25 ml Freund's complete adjuvant (Difco Laboratories, Detroit, Michigan) emulsified with 10% water. Group CNS, the same as CF but including 35 mg of homogenised adult rat spinal cord, 0.21 mg bovine serum albumin (crystallised and lyophilised, Sigma, USA) or 0.21 mg poly-L-lysine (hydrobromide, Type I, molecular weight 41,000, Sigma, USA). Groups BPL, AbL and PyL, the same as groups BP, Ab or Py, respectively, plus a mixture of CNS lipids consisting of 0.16 mg sulphatides, 0.7 mg cerebroside, 0.85 mg cholesterol, gangliosides corresponding to 0.02 μ mol sialic acid, and phospholipids corresponding to 2.5 μ mol lipid phosphorus. Each of the preparations was sonicated for 3 min at 20 kHz in a Branson sonifier before injection.

*Individual lipids in group CF were expressed per 100 mg total lipids. In all the other groups the figures represent percentage differences between means, taking the values for group CF as 100%. *P* was calculated by Student's *t* test for non-correlated samples.

†Significant at *P* < 0.02.

‡Significant at *P* < 0.05.

§Significant at *P* < 0.001.

in the different groups were consistent with the changes shown in Table 1. Thus, we found decreases of 48.4% ($P < 0.001$) in group CNS, 9.8% (not significant) in group BP, 51.8% ($P < 0.001$) in group BPL, 0.7% (not significant) in group Ab, 38.4% ($P < 0.02$) in group AbL, 11.1% (not significant) in group Py and 37.1% ($P < 0.02$) in group PyL, (control value: $25.0 \pm 1.2 \mu\text{mol}$ glycolipid hexose per 100 mg of total lipid).

Our observations indicate that the presence of PBP in the injection mixture, necessary to induce the neurological disturbances observed in EAE, is not specifically required to induce the lipid alterations in the CNS. On this basis, EAE classically induced with whole CNS seems to be a composite result of different effects: the phenomena related to the paralytic symptoms elicited by the PBP, and the alterations induced by the lipidic components present in the CNS tissue, independent of clinical symptoms. Such differences between EAE induced by whole CNS and by PBP have already been observed. A demyelinating, or myelination inhibition, factor is present in serum of EAE animals sensitised with whole CNS but it is not consistently induced when PBP is used as encephalitogen^{8,9}. The antibody to cerebroside is responsible both for the disturbances in the sulphatide metabolism and the demyelination effects observed in cord tissue cultures exposed to EAE serum⁸. Thus, it seems likely that our observations *in vivo* may be caused by similar phenomena, as suggested⁶. Inoculation of rabbits with cerebroside, adjuvant and albumin resulted in perivascular infiltration in the spinal cord but not paralysis¹⁰; these findings seem to be closely related to those recently observed in EAE.

On the other hand, the surprising results obtained with poly-L-lysine suggest that the basic nature of the protein injected is closely related to changes in the content of anionic lipids. This should lead to a study of the effects of the injection of other basic, acidic and neutral proteins on the lipid composition of the CNS. Recent findings¹¹⁻¹⁴ regarding the highly specific interaction of the myelin basic protein molecule with negatively charged lipids such as sulphatides at the air-water interface are relevant. If such interactions and molecular arrangements are also occurring *in vivo* in the CNS, the alterations observed may be interpreted as the consequence of specific disruptive responses directed towards some of the interacting molecules or particular structural assemblies.

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BRUNO MAGGIO
FEDERICO A. CUMAR

Departamento de Química Biológica,
Facultad de Ciencias Químicas,
Universidad Nacional de Córdoba,
Córdoba, Argentina

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Marijuana, absinthe and the central nervous system

THERE are striking similarities between the psychological actions of the liqueur absinthe¹ and the experiences frequently reported by users of marijuana². We have therefore compared the properties of thujone and tetrahydrocannabinol (THC), which are believed to be the active principles of *Artemisia absinthium* and *Cannabis sativa*, respectively. Both substances are terpenoid, derived from the essential oils absinthol and cannabinal, and are formed by similar biosynthetic mechanisms^{3,4}.

The effects of absinthe have been known since the last century, but thujone has been conspicuously absent from recent lists of psychotropic plant products. It has been traditionally grouped with two $\text{C}_{10}\text{H}_{16}\text{O}$ isomers, camphor and menthol⁵, and classified as a convulsant poison. The molecular geometry of these three compounds is so different, however, that it is difficult to believe that, at low doses, they interact specifically with the same pharmacological receptors. At large doses, it is always possible that they exert similar, less specific actions by virtue of common physicochemical properties.

Thujone and THC have similar molecular geometry and similar functional groups available for metabolism. This close geometrical resemblance is illustrated in Fig. 1, in which the bonds common to both molecules are drawn as bold lines. The similarities include the gem-dimethyl groups at C_8 , the C_7 methyl groups, the bonds connecting carbons 8, 4, 3, 2 and 1 of THC and 8, 5, 4, 3 and 2 of thujone, the α hydrogen at C_4 in THC and the cyclopropyl 5-6 bond of thujone and the 4-5 bond in THC and the 1-5 bond in thujone. Finally, although there is

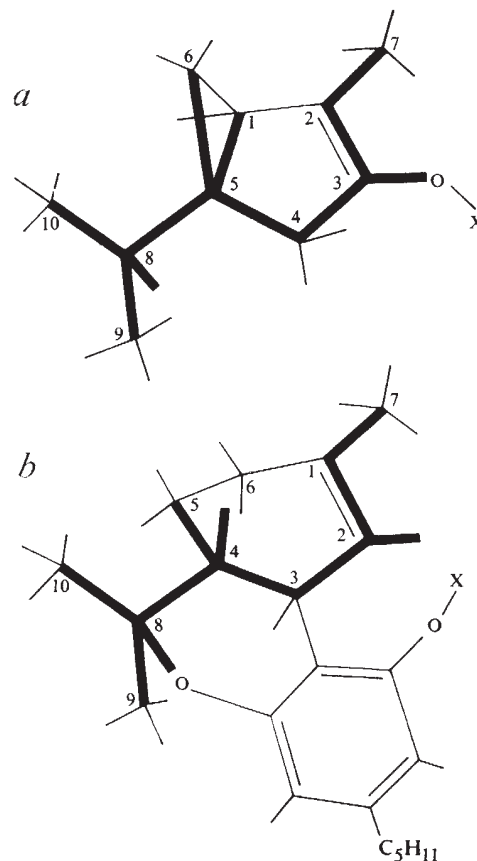


Fig. 1 Structural formulae of thujone-enol (a) and $\Delta^1,6$ -THC (b). Bonds common to both molecules are drawn as bold lines. X indicates the site of the receptor with which the oxygen of the thujone molecule or the hydroxyl group of the THC molecule may react. See text for details.

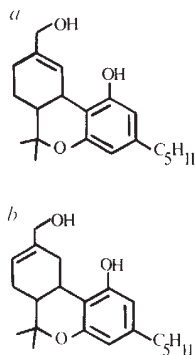


Fig. 2 Products of metabolism of $\Delta^{1,2}$ -THC (a) and $\Delta^{1,6}$ -THC (b).

no direct correspondence between the oxygen of the thujone molecule and the hydroxyl group of THC, it seems possible that both react with a common site of a pharmacological receptor, such as that indicated by X in Fig. 1, without changing the orientation or relative position of either molecule.

These oxygen atoms are likely to be the principal pharmacological binding sites because the primary metabolites from $\Delta^{1,2}$ -THC (ref. 6) and $\Delta^{1,6}$ -THC (ref. 7), both of which are physiologically active, are products of oxidation at C_7 (Fig. 2). Indeed, these metabolites have been suggested as the actual psychotomimetic agents in marijuana⁷.

The $\Delta^{2,3}$ -enolic form of thujone contains an allylic group at $C_{3,2,7}$ which is analogous to $C_{2,1,7}$ in $\Delta^{1,2}$ -THC; both systems should be available for similar oxidative reactions. In fact the electron-releasing ability of oxygen in the thujone enol should increase the rate of hydrogen removal at C_7 relative to a comparable reaction in THC (ref. 8). That thujone can exist in the $\Delta^{2,3}$ -enol form is shown by its reaction with permanganate, a process known to involve enols⁹. Since the reaction affords an almost quantitative yield of the keto-acid¹⁰ shown in Fig. 3 there is little doubt that the $\Delta^{2,3}$ -enol form is involved. Further, the existence of enols in biological systems is well documented¹¹.

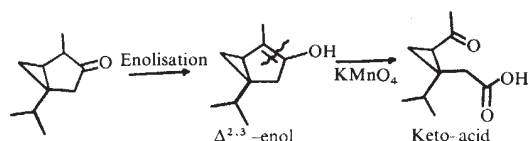


Fig. 3 Permanganate oxidation of thujone by way of the $\Delta^{2,3}$ -enol.

We propose therefore that both thujone and THC exert their psychotomimetic effects by interacting with a common receptor in the central nervous system. Topologically, this receptor should have a binding site for interaction with oxygen, a planar region to accommodate the allyl system, and pockets or cavities in which the alkyl and hydrogen substituents common to both drugs would fit. This hypothesis suggests new experimental approaches to study the pharmacology and toxicology of these and related compounds. A common mechanism of action for THC and thujone is also interesting from a historical and sociological point of view.

J. DEL CASTILLO
M. ANDERSON

Laboratory of Neurobiology
School of Medicine, UPR,
San Juan, Puerto Rico 00905

Department of Chemistry,
University of Idaho,
Moscow, Idaho 83843

G. M. RUBOTTOM

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Decrease in 17β -oestradiol receptor in brain of ageing rats

WE have shown^{1,2} that the administration of 17β -oestradiol to ovariectomised female rats induces acetylcholinesterase (AChE; EC 3.1.1.7) in the cerebral hemisphere and cerebellum, and that this induction decreases with increasing age. The greatest induction occurs in the immature rat, and there is no induction in the old. As 17β -oestradiol receptors are present in rat brain^{3,4}, we suggested that the impairment of AChE induction in the brain of old rats may be due to a decrease in the level of this receptor. This study was designed to test our hypothesis, and the data show for the first time that it may be correct.

Immature (7 week), adult (44 week) and old (108 week) female Wistar rats maintained in the rat colony were used. The rats of the three age groups were bilaterally ovariectomised and reared on tap water and standard diet for 21 d to ensure complete disappearance of oestradiol from the blood as described earlier^{1,2}. They were killed on day 22 and the cerebral hemispheres were removed to prepare a 10% homogenate (w/v) in Krebs-Ringer bicarbonate buffer, pH 7.4, containing 2.8 mmol glucose.

The procedure followed for characterising the oestradiol-receptor complex of the cytosol was adopted from that of Wong and Burton⁵ for the hydrocortisone-receptor in the nuclei of placenta. 3H -oestradiol (0.2 μ Ci) (Radiochemical Centre, Amersham) was added to 1.0 ml aliquots of homogenate and the samples were kept on ice for 30 min with occasional stirring. They were then incubated at 37°C for 10 min in a water bath shaker after which 5.0 ml of 1.5×10^{-3} M $MgCl_2$ were added to each. Then they were kept in ice for 15 min and centrifuged at 700g for 15 min at 0°C. The pellet was resuspended in an equal volume of $MgCl_2$ and centrifuged as before. Both the supernatants were pooled and centrifuged at 14,500g for 1 h. The supernatant thus obtained was fractionated through a 11 $\times$ 180 mm column of Sephadex G-25-80 (Sigma) at 2°C using an elution buffer of 0.6 M KCl, 10 mmol Tris and 1.5 mmol EDTA, pH 8.0. Fractions (1.0 ml) were collected and a 0.1 ml aliquot of each fraction was applied on 2 $\times$ 2 mm Whatman No. 1 filter and dipped in 12.0 ml of scintillation fluid (PPO: POPOP; 4:0.4 in 1.0 l toluene). The radioactivity was counted in a Packard Tri-carb liquid scintillation spectrometer (Model 3003). The concentration of protein (μ g ml⁻¹) was measured according to the method of Sutherland *et al.*⁶. The averages of the data collected from four or five rats of each age are given in Fig. 1.

Figure 1 shows a sharp peak and a high level of oestradiol-protein complex in the cytosol of the immature rat as compared to those of the adult and the old rat. The peak for the radioactivity corresponds to the protein peak. It is also noteworthy that the peak for the labelled-complex gets diffused in the adult and the old indicating a loss of specificity or affinity of the receptor protein of the cerebral hemisphere which binds to oestradiol.

So far, the only enzyme of the cerebral hemisphere which

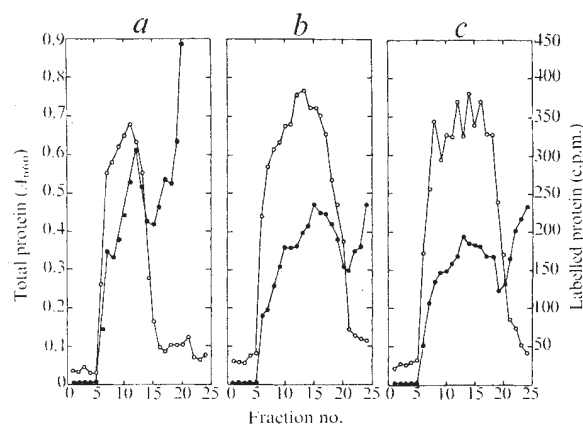


Fig. 1 Elution profile of labelled protein (^3H -oestradiol-protein complex) (●) and total protein (○) of the cytosol of the cerebral hemisphere of, a, 7; b, 44 and, c, 108-week-old ovariectomised rats by Sephadex G-25-80 chromatography.

is known to be induced by 17β -oestradiol is AChE. Our data do not show directly that it is this 17β -oestradiol-protein complex which mediates the induction of AChE but the positive correlation between the degree of induction of AChE and of the level of 17β -oestradiol-protein complex is of great significance.

The lag period for the induction of tyrosine aminotransferase in the liver of the rat is known to increase with age⁷. We have shown that the induction of malate dehydrogenase⁸ and glutamine synthetase⁹ of the liver of rats by cortisone is impaired in old age. This impairment is similar to that of AChE of the brain^{1,2}.

These data show that the impaired induction of certain enzymes by steroid hormones in old age may be due to the depletion of specific receptor proteins in the cytosol of target cells. The receptor being a protein, its synthesis may be under the control of a unique gene whose expression may be regulated by a specific factor (for example, a hormone) that decreases after development. Also, the decrease in the level of a specific receptor with high affinity, and the possibility of the appearance of certain proteins having unspecific affinity for the hormone may account for the diffused nature of the receptor peak in older rats. The possibility, however, of the appearance of molecules which may repress specific genes of these enzymes in old age cannot be ruled out. This is consistent with the view that programmed changes in the activities of genes begin after fertilisation and continue throughout the life span^{10,11}. This may account for the sequential changes in the enzyme pattern as observed by us for cytoplasmic alanine aminotransferase of the liver of the rat during its life span (M.S.K. and S.K.P., unpublished). This may, in turn, account for differentiation, development and senescence of an organism.

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M. S. KANUNGO
S. K. PATNAIK
OMANAND KOUL

Biochemistry Laboratory,
Department of Zoology,
Banaras Hindu University,
Varanasi 221005, India

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Interdigitated repeated sequences in bovine satellite DNA

MOST eukaryote genomes contain some highly repeated DNA sequences. DNA hybridisation kinetic studies indicate that the smallest repeating unit of highly repetitious DNA is around 300 base pairs¹ but direct sequence analysis of highly repeated DNA has demonstrated a simple basic repeating unit of 4–12 nucleotides^{2–5}. It has been postulated that the repeated sequences have been generated by successive replications of some primary simple sequence^{1,6,7}. The discrepancy between sequence data and kinetic analysis may be explained by noting that the rate of reassociation is a function of length⁸; this data is consistent with a model of internal heterogeneity in the repeated, simple DNA sequence units^{9,10}. Although the hybridisation and sequence analyses have been successful in establishing certain features of reiterated DNA, these methods are not well suited to detect and study infrequent but regularly repeated DNA sequences interdigitated within the dominant short repeating units. The existence and nature of such interdigitated repeats are important to our understanding of the mechanism by which repeated DNA is generated. We have used site-specific endonucleases (restriction enzymes) to show that mutational events within simple sequence DNA may have been coupled with cycles of discrete replications.

If a stretch of DNA were comprised of only a simple repeated sequence which contains the restriction endonuclease recognition site, it would be cleaved to fragments the size of the simple sequence. Conversely, if the simple sequence lacked the recognition site, it would be refractory to digestion. The results of digesting bovine DNA with the sequence-specific endonuclease isolated from *Haemophilus influenzae* Rd (endo R·Hind II+III)^{11,12} and analysing the digestion products by polyacrylamide gel electrophoresis have already been reported^{13–16}. This enzyme produces an expected continuous distribution of DNA fragments; superimposed on this distribution are at least twelve distinct, specific fragments ranging in size from 1.2×10^3 to 2×10^6 , and reiteration frequencies from 2,000 to 140,000 (in a previous report¹⁴ the smallest fragment, XII, was assigned a molecular weight of 7.5×10^4 ; our more recent data indicate it is 1.2×10^5). These twelve size classes of fragments could only be produced if the fragment termini containing the restriction site occurred at regularly spaced repeated intervals.

To ascertain whether there are additional interdigitated repeats besides those detected by the *Hind* digestions, bovine DNA was hydrolysed with endo R·Hae (the restriction enzyme from *Haemophilus aegyptius*¹⁷). Approximately 30 discrete bands were produced (Fig. 1, third lane). These fragments were generally smaller than those observed in a *Hind* digestion (Fig. 1, first lane). Most of the low molecular weight *Hae* fragments (Fig. 1, region 27–33) increased in size progressively by steps of approximately 10 base pairs up to a fragment length of about 100 base pairs. This step increase is in the size range of the simple repeating sequences found in other organisms^{2–5}. Although the digestion profile in the region of the larger fragments becomes considerably more complex, it is tempting to extrapolate that incremental size increases are by some variable multiple of a 10 base repeat.

The relationship between the two sets of fragments generated by *Hind* and *Hae* was determined. Figure 1 (first, second and third lanes) shows a comparison of the digestion profile produced by a *Hind* plus *Hae* digestion with the profiles produced by each enzyme alone. This comparison showed that all of the *Hind* fragments were abolished by *Hae*.

To determine whether the *Hae* fragments are contained within the *Hind* fragments, preparative quantities of several specific *Hind* fragments were eluted from gels¹² and analysed after secondary digestion with *Hae* (Fig. 1). One obvious aspect of these results is that the sum of the molecular weights of the *Hae* fragments approximately equals the molecular weight of their respective parental *Hind* fragments. But, in the case of *Hind* fragments V and VIII the molecular weights of the derived *Hae* fragments add up to twice that of their respective parents, suggesting that *Hind* V and VIII are each comprised of two fragments. Cross contamination between *Hind* fragments can be ruled out as an explanation for this observation, since control experiments in which *Hind* fragments were re-electrophoresed on gels, indicated this to be less than 10%. Similarly, the sum of the *Hae* fragments does not equal the molecular weight

of *Hind* fragment III unless *Hae* fragment 6 is added twice as suggested by its relative intensity. Thus, it is concluded that in each case the *Hae* fragments are comprised of the same nucleotide sequences as their respective *Hind* fragment.

A second point is that a number of the *Hae* bands are common to several different *Hind* fragments (in this sense 'common' refers to fragments with the same electrophoretic mobility). For example, many of the low molecular weight *Hae* fragments in the region 19 to 30 inclusive are common to *Hind* fragments IV, V, VI+VII, and VIII (Fig. 1). Similarly, *Hae* fragments 19 and 20 are common to *Hind* fragments II, III, V, VIII, and IX, and *Hae* fragment 16 is common to *Hind* fragments V, VI+VII, and VIII, and there are other cases. These instances of the same *Hae* fragment occurring within several different *Hind* fragments suggest considerable internal sequence similarity among some of the *Hind* fragments.

The juxtaposition of individual fragments can be discerned by identifying DNA regions containing a *Hind* site adjacent to a *Hae* site. Such a configuration will yield fragments not shared by the *Hind*, *Hae*, and combined *Hind* plus *Hae* digestion profiles. One such overlap has been identified in *Hind* fragment XII by virtue of fragments 24

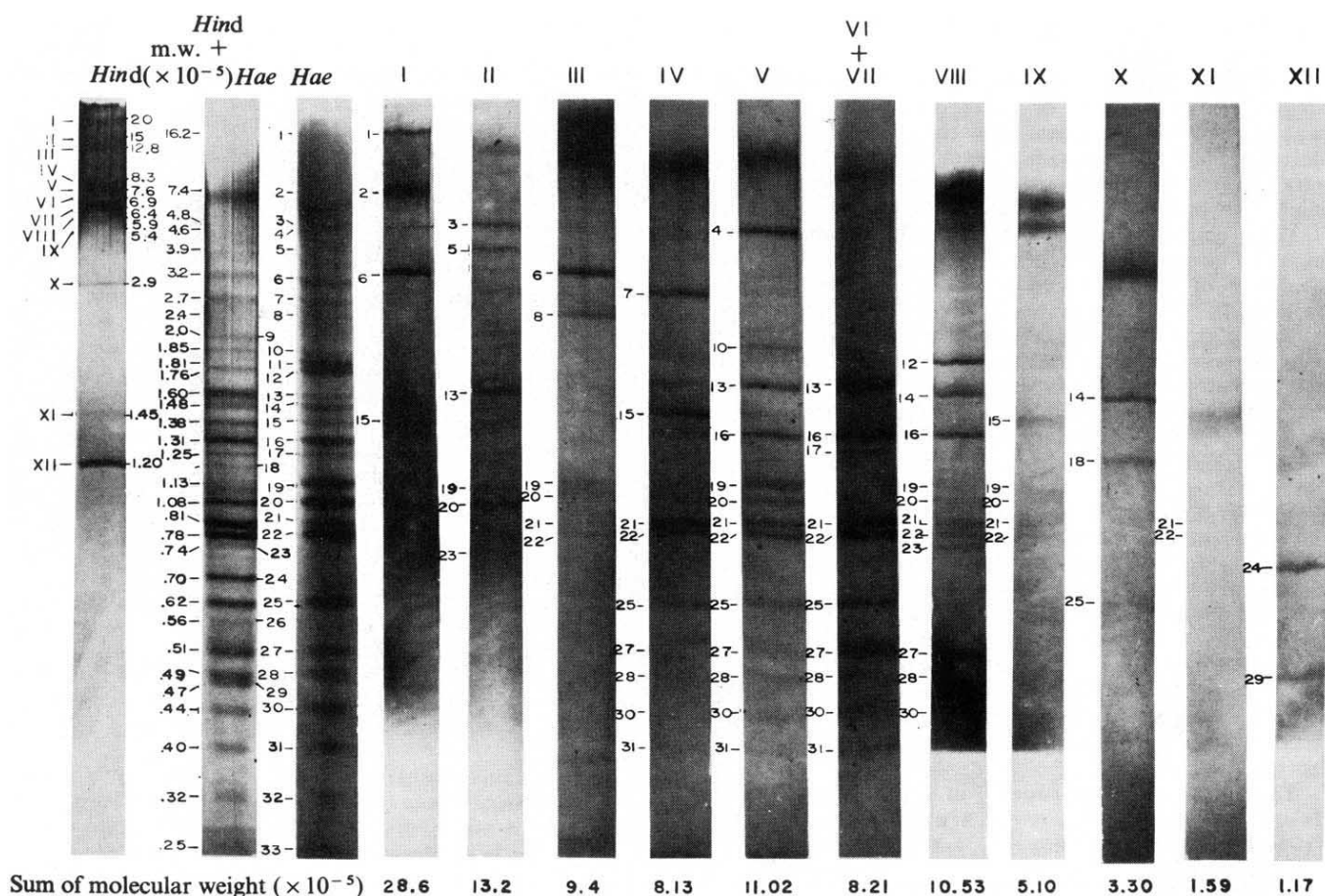


Fig. 1 *Hae* cleavage products of repeated *Hind* fragments in bovine DNA. The first three lanes are digests of total bovine DNA: first, *Hind* alone; second, *Hind*+*Hae*; third, *Hae* alone. Molecular weights were determined by coelectrophoresis with *Hind* fragments of $\Phi 80h$ DNA¹⁷. The remaining lanes are secondary digests prepared as follows. Total bovine DNA was hydrolysed with *Hind* and fractionated by electrophoresis on preparative 2% acrylamide-0.5% agarose slab gels at 4°C and 200 V (ref. 14). Individual bands were cut from the gel. The DNA was electrophoretically eluted and after concentration and dialysis was hydrolysed with *Hae*. Subsequent electrophoresis was on 4% acrylamide-0.5% agarose gels at 4°C and 200 V. The *Hind* fragments used as substrate for the secondary *Hae* digestion are given in Roman numerals at the top of each lane. Arabic numbers are assigned to the bands produced by hydrolysis with *Hind*+*Hae* or *Hae* alone. In some cases, faint *Hae* fragments occurring in *Hind* profiles are not labelled (for example, a *Hae* fragment in *Hind* fragment I corresponding to *Hae* fragment 3). There is a small amount ($\leq 10\%$) of cross contamination of *Hind* fragments in the preparative gels, and this is the most likely source of these faint bands. *Hind* fragments VI+VII have been presented together because they have not been adequately resolved on these gels. *Hind* fragment VII, however, is 15 times more abundant than *Hind* fragment VI¹⁴; thus, *Hae* fragment 17 probably originates from *Hind* fragment VI.

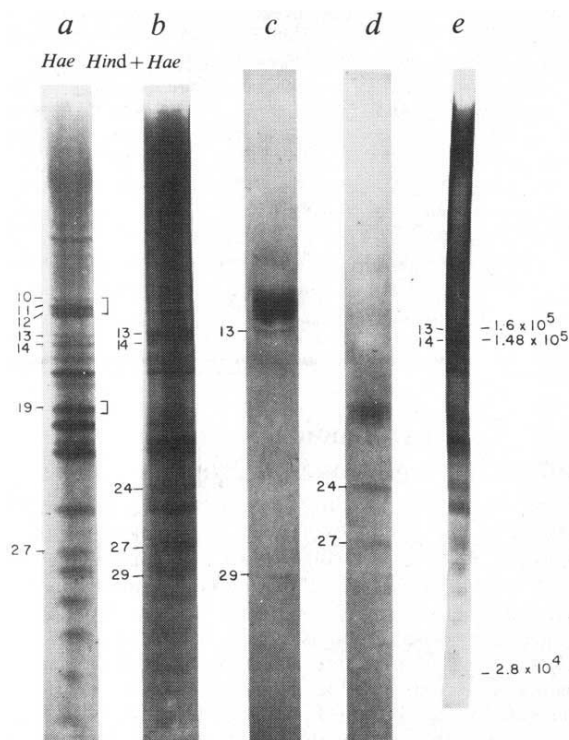


Fig. 2 Determination of the partial order of *Hae* fragments within and overlapping *Hind* fragments VII and XII. Bovine DNA (1 mg) was hydrolysed with *Hae* and electrophoresed on a 4% acrylamide-0.5% agarose slab gel. The regions of the gel indicated by brackets were cut out, and the DNA was eluted as described in Fig. 1. DNA from the upper bracketed region and from the lower bracketed region, containing *Hae* unique fragments 11 and 19, respectively (see text), were each further hydrolysed with *Hind* and electrophoresed in 4% acrylamide-0.5% agarose (lanes c and d, respectively). Bovine DNA hydrolysed with *Hae* (lane a) and *Hind*+*Hae* (lane b) were included as controls. The numbers conform to the band labelling system utilised in Fig. 1. Lane e is an *EcoRI* hydrolysate of *Hind*+*Hae* digested bovine DNA. The molecular weights of the products were determined as indicated in Fig. 1. See text and Fig. 1 for further discussion.

and 29 (Fig. 1, lane XII) which are present only in the combined *Hind* plus *Hae* digest. Nearest neighbour information between *Hind* fragment XII and other *Hind* fragments can be detected by identifying those *Hae* fragments which on *Hind* digestion give rise to 24 or 29 (Fig. 3). *Hae*

fragment 11, which is not found in the combined *Hind* plus *Hae* digest, when subsequently digested with *Hind* yields fragments 13 and 29, in addition to undigested material (Fig. 2, lane c). Fragment 13 is found in the *Hae* digests of *Hind* fragments II, V, and VI+VII. Of these potential *Hind* fragments as neighbours to fragments XII, only fragment VII seems likely. This deduction is based on earlier evidence in which we determined that the buoyant density of sheared DNA containing *Hind* XII was the same as that of sheared DNA containing *Hind* fragment VII, and was of a significantly different density than that of sheared DNAs containing the other *Hind* fragments as nearest neighbour candidates¹⁴.

The neighbour on the other side of *Hind* fragment XII can be determined by similar reasoning. *Hae* fragment 19 which is not found in the combined *Hind* plus *Hae* digest, when subsequently digested with *Hind* yields fragments 24 and 27 (Fig. 2, lane d). As noted above, fragment 24 is unique to *Hind* fragment XII. Fragment 27 is found in *Hind* fragments IV, V, VI+VII and VIII. From the buoyant density data described above, fragment VII is the most likely candidate as the neighbour to XII. From this it is concluded that fragments VII and XII occur adjacent to one another, in an alternating tandem fashion. Other cases of overlapping regions which can be discerned by comparing the *Hind*, *Hae*, and combined *Hind* plus *Hae* digestion profiles will not be discussed here since they are less well resolved.

This conclusion that *Hind* fragments VII and XII alternate with each other is consistent with the observation of Botchan¹⁶ that purified bovine satellite I DNA, when digested with *Hind*, gives rise only to products corresponding to our fragments VII and XII. We have observed that the endo *R-EcoRI* restriction site¹⁸ discussed in the work of Botchan in fact occurs in *Hae* fragment VII-13, because it disappears in a combined *Hind* plus *Hae* plus *EcoRI* digest and gives rise to fragments of molecular weights 0.3×10^5 and 1.48×10^5 (Fig. 2, lane e, and Fig. 3). Similar conclusions have also been reached by P. Philippsen (personal communication) with the further finding that in addition to fragments 24 and 29 there is a very small *Hae* fragment which is produced from the centre of *Hind* XII.

Having obtained these patterns with *Hind* and *Hae*, it was of interest to ascertain whether any other restriction enzymes yielded equally dominant repeat patterns. To test this, a series of experiments was carried out in collaboration with Dr R. J. Roberts. A *Hind* plus *Hae* digest of bovine DNA was subjected to digestions with each of ten additional restriction endonucleases which were provided by Dr Roberts: *Hpa* I and II, *Hha* I, and *Hph* I (ref. 19);

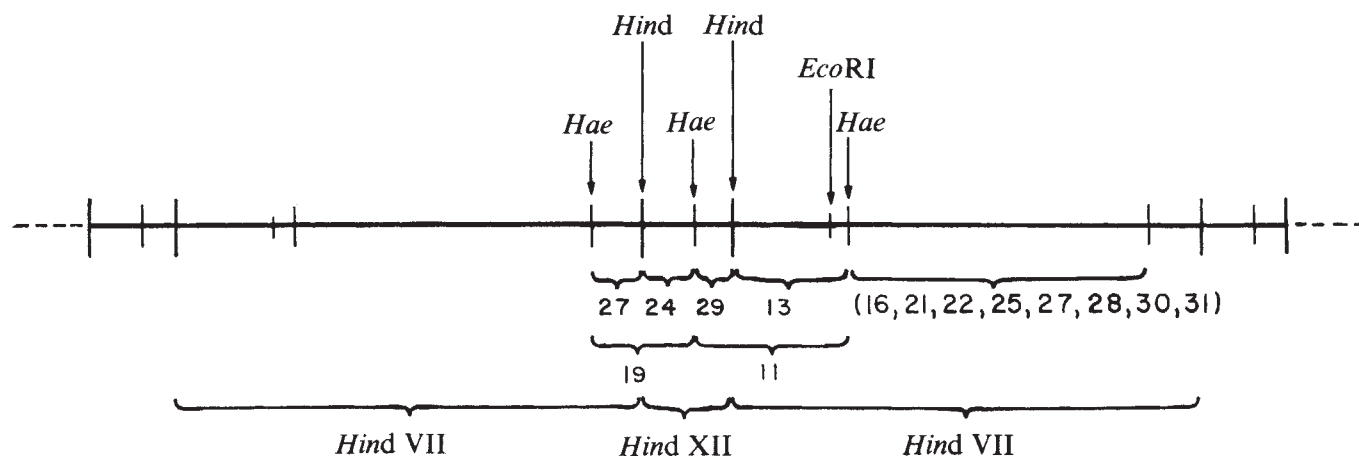
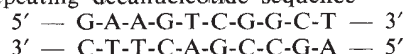


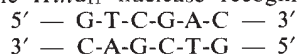
Fig. 3 Schematic representation of the arrangement of *Hind* fragments VII and XII and their internal *Hae* fragments. See the text and Figs 1 and 2 for further descriptions.

Alu I, isolated from *Arthrobacter luteus* (R. J. Roberts, unpublished); *Sma*, isolated from *Serratia marcescens* (C. Mulder, unpublished); *Hga* I, isolated from *H. gallinarum*²⁰; *Sal* I, isolated from *Streptomyces albus* G. (R. J. Roberts, unpublished); *Sac* I, isolated from *Streptomyces achromogenes* (R. J. Roberts, unpublished); and *Xan* I, isolated from *Xanthomonas amaranthicola* (R. J. Roberts, unpublished). The majority of the *Hind* plus *Hae* fragments were not abolished by these additional restriction enzymes, thus indicating that the repeats demonstrated by the rather frequent occurrence of the *Hae* cleavage site are quite sequence dependent (data not shown).

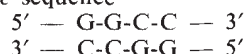
The internal DNA sequence within each of the fragments need not, *a priori*, contain reiterated DNA, but we have shown that most of the twelve *Hind* fragment size classes can be derived from positions in isopycnic gradients also known to contain satellite DNA (ref. 14). It has also been shown that at least two of the *Hind* fragments reanneal with the kinetics of a simple sequence¹⁶. Therefore, we postulate that the repeated *Hind* and *Hae* nuclease restriction sites differ only by one or a few bases from some primary repeating sequences. For example, in the hypothetical primary repeating decanucleotide sequence



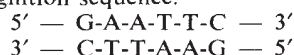
a single transition in the eighth position could yield one of the two possible *Hind*_{II} nuclease recognition sequences¹¹,



(All twelve *Hind* fragments except for fragment VIII are bounded by two *Hind*_{II} sites; unpublished results.) Similarly, a single transition in the tenth position of this decanucleotide yields the *Hae* sequence



(K. Murray and R. Roberts, personal communication). A transversion in position 4 of the decanucleotide would yield the *Eco*RI recognition sequence.



(ref. 18) which occurs in fragment 13 (see Fig. 3).

The data presented in this report demonstrate the existence of different orders of interdigitated repeats within the satellite fraction of DNA. These results support the idea, advanced previously by others^{1,6,7}, that multiple rounds of replication, and/or unequal crossing over are separated by intervening mutational events.

Sequence analyses now in progress should serve to establish the precise relationship between the *Hind* and *Hae* nuclease recognition sites and the postulated primary simple repeat.

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SAMUEL L. MOWBRAY

Department of Biological Sciences,
Smith College, Northampton,
Massachusetts 01060

SUSAN A. GERBI
ARTHUR LANDY

Division of Bio-Medical Sciences,
Brown University,
Providence, Rhode Island 02912

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Super dense carotenoid spectra resolved in single cone oil droplets

CONE visual receptors in reptiles and birds contain brilliant yellow, orange or red oil droplets, 3-6 μm in diameter, through which light must pass before reaching the visual pigment. The possible role of these brightly coloured organelles in colour vision has been disputed¹⁻⁶. Their colours are thought to be caused by various carotenoids whose characteristically peaked absorption spectra in organic solvent extracts of whole retinas were first reported by Wald and Zussman⁷. In spite of this, subsequent workers have failed to identify the dramatic carotenoid spectra⁸ in individual oil droplets by direct methods of microspectrophotometry⁹⁻¹³. Instead, structureless cutoff absorbance curves reminiscent of commercial colour filters have been found to truncate at about one absorbance unit without any evidence of carotenoid spectral fine structure (Fig. 1).

It is not known why individual micromasurements do not substantiate the results of extraction. Neither is it understood, therefore, precisely how individual oil droplet colours are contrived. The flat-top and cutoff spectral character evident *in situ* might easily be accounted for by spectral overlap in various carotenoid mixtures. Alternatively, these properties might be a manifestation of instrumental limitations of microspectrophotometers¹⁴. Although our absorbance measurements on single oil droplets were done with some care, passing 1-2 μm diameter circular microbeams through their centres to reduce leakage of incident light around the sides, stray or scattered light might still dominate these measurements if the absorbance was very high¹⁴. Such artefacts could yield a plateau throughout the spectral region where true specimen density exceeds instrument capability.

To pursue this problem we initially improved our microspectrophotometer¹³ to record higher absorbances up to 2.7 but found the oil droplet spectra to plateau again at this new limit. Having achieved a practical limit for high-density microspectrophotometry, still without resolving spectral structure, we then turned to Lambert's law which suggests that absorbance might be reduced to measureably low values by reducing the optical pathlength. To accomplish this we squeezed oil droplets from freshly isolated retinas in a screw press under microscopic observation. We also air dried collections of oil droplets from distilled water to cause surface tension flattening. Still further thinning was achieved by exposure of air dried preparations to organic solvent vapours of hexane, pyridine, benzene or chloroform. We achieved only limited success with each method as we could only begin to see evidence of spectral fine structure. Moreover, the spectrum changed spontaneously on exposure to air and we could not achieve the quantitative results we now describe.

Retinas were removed from excised fresh turtle eyes (*Pseudemys scripta* or *Chelonia mydas*) in Ringer solution and were dropped into a small Petri dish of distilled water. After 30 min, a sediment of oil droplets released from lysed cells could be seen with a dissecting microscope. A drop of this sediment was sucked into a Pasteur pipette and transferred to a microscope

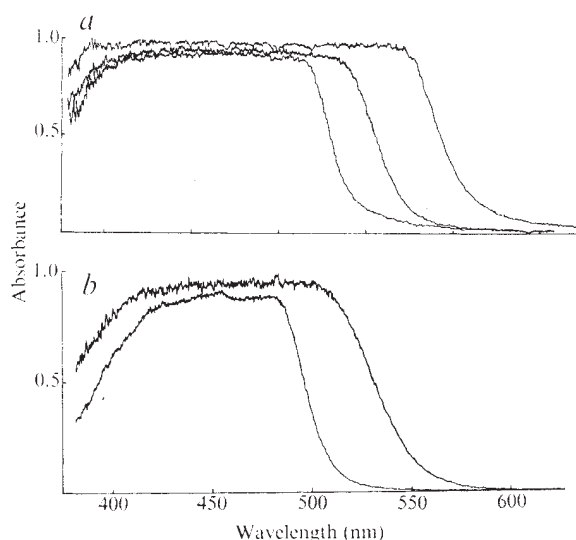


Fig. 1 Spectra of individual cone oil droplets recorded directly in excised turtle retinas with a microspectrophotometer. Each spectrum shows a plateau near one absorbance unit. *a*, The swamp turtle, *Pseudemys*. From left to right, yellow, orange and red droplet spectra. *b*, The sea turtle, *Chelonia*. From left to right, yellow and orange droplet spectra.

slide. This was viewed at 650–1,000 $\times$ magnification in an inverted microscope equipped with a Leitz micromanipulator. Individual oil droplets were then identified and their diameters measured. A 3–4 μ m tip micropipette attached to a pressure injection syringe was brought into contact with the oil droplet and a tiny drop of mineral oil (Squibb, USP C₈₋₁₂ hydrocarbon) expressed from the tip. As the two droplets fused, the pressure was quickly relieved and the pipette tip raised, leaving the new (20–50 μ m diameter) coalesced sphere attached to the glass slide with a swirling mixture of colour in its midst. Complete mixing, as evidenced by colour homogeneity, occurred within 10 s. The diameter of the new diluted droplet was measured and microspectrophotometry done using a 5 μ m microbeam passed through its centre. This produced resolved spectral structure in each case (Fig. 2).

Using the conservation law, $C_1V_1 = C_2V_2$, and the Beer–Lambert law, $D = \epsilon cl$, where c is concentration, V is volume, D is optical density, ϵ is extinction coefficient, l is optical path-length and the subscripts 1 and 2 refer to the original and diluted droplet respectively, it is easy to see that the absorbance of pigment in the original droplet was

$$D_1 = D_2(R_2/R_1)^2 \quad (1)$$

if the droplets were spheres of radius R measured from their plan view. Histologically, the original droplets are spheres but as the diluted droplets are never seen in cross section, the above formula might not be applicable should the enlarged droplet flatten to become an ellipsoid or hemiellipsoid of revolution instead of a sphere. It can be shown, however, that these non-spherical forms yield self-cancelling effects on the dilution factor and pathlength leading to precisely the same formula as for spheres. Although it could be speculated that Beer's law might not apply at such high pigment concentration, we have evidence to the contrary, for the shape of the spectrum of the diluted pigment (Fig. 2) at very long wavelength provides a satisfactory fit to the long wave part of the original spectrum (Fig. 1) when scaled by the factor D_1/D_2 obtained from equation (1).

Thus, our experiments yield several conclusions (Fig. 2, Table 1). Each pigment is exceedingly dense; indeed, such high recorded absorbances are unknown to us in any other cellular structure. The red and orange droplets of *Pseudemys* have the same spectral shape, that is, their pigments are identical. Their spectrum appears to match one of the extracts, that of astaxan-

thin^{7,8}, while that of the yellow droplet is similar to zeaxanthin or xanthophyll^{8,15}.

We conclude that in spite of similar composition the orange droplet cuts off at lower wavelength than the red simply because of its nearly tenfold lower concentration and optical density. The absorbance of the 6 μ m diameter red droplet, however, is 50–90 absorbance units. At peak, this droplet may transmit only 10⁻⁹⁰ of light incident on it to the cone outer segment, that is, it cuts out 'completely' at peak. Preliminary results on the red droplet of birds also indicate extremely high optical density.

Table 1 Range of peak absorbance found in individual oil droplets by the method described in text

	R	O	Y
<i>Pseudemys</i>	50–90	6–9	12–18
<i>Chelonia</i>		4–7	3–4

R, red; O, orange; Y, yellow.

Using its published extinction coefficient in hexane¹¹ it is possible to compute a concentration of about 1 M for astaxanthin in the *Pseudemys* red droplet. Using reasonable values for its partial molal volume, it can be concluded that astaxanthin itself occupies over half the space of the red oil droplet containing it. Indeed, if a red droplet is scratched with a micropipette, the material behaves like crayon or a viscous wax. Its failure to crystallise in these circumstances is probably consistent with the presence of a mixture of long chain *cis* and

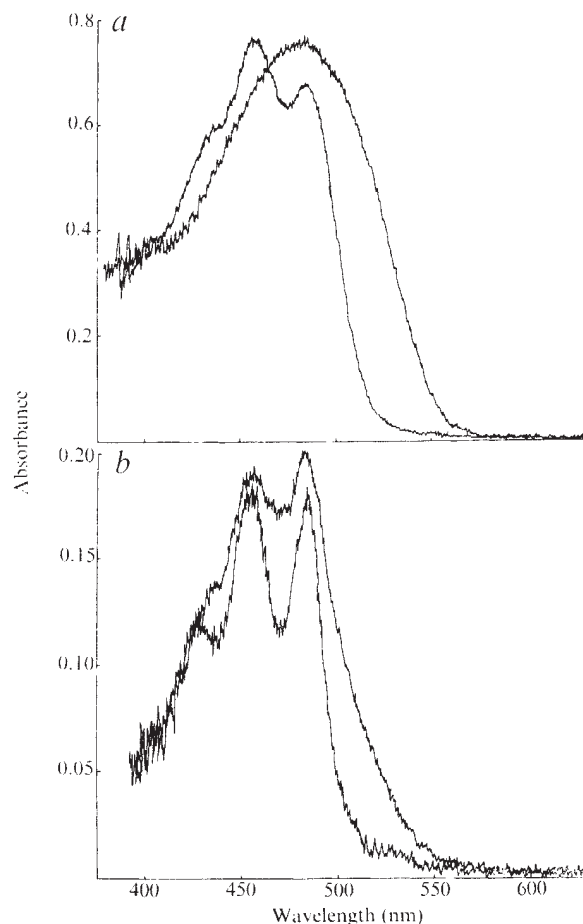


Fig. 2 Spectra of individual turtle oil droplets after enlargement and dilution with mineral oil. *a*, *Pseudemys*, three-fingered spectrum of yellow droplet (left hand trace) singly peaked spectrum of orange and red droplets (right hand trace); *b*, *Chelonia*, sharply fingered spectrum of yellow droplet (lower trace); orange droplet (upper trace).

trans isomers that retain a hindered fluidity or domain structure.

In *Chelonia* a somewhat different picture emerges. No red droplets are present but both orange and yellow diluted droplets have a three-fingered fine structure. They are not quite a match, however, and we can only say that each droplet seems to contain a single pigment of high absorbance, though not as imposing as those of *Pseudemys*.

The oil droplet pigments of both turtle species are sufficiently dense to strongly alter the spectral distribution of light striking the photosensitive visual pigments. They must therefore play a role in colour vision and like the visual pigments themselves¹⁶, only a single pigment species is present in each cell.

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P. A. LIEBMAN
A. M. GRANDA

Department of Anatomy,
University of Pennsylvania,
Philadelphia, Pennsylvania 19174
and Department of Psychology,
University of Delaware,
Newark, Delaware 19711

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Regulation of RNA polymerase II activity in a mutant rat myoblast cell line resistant to α amanitin

A MUTANT cell line, Ama102, resistant to the cytotoxic effects of α amanitin, the specific inhibitor of RNA polymerase II (refs 1 and 2), has been selected³ from cultures of Yaffe's⁴ rat skeletal muscle myoblast cell line, L₆, treated with mutagen. Ama102 cells have similar doubling times for growth in the absence or presence of 3 $\mu\text{g ml}^{-1}$ α amanitin whereas the Ama⁺ parent does not survive in this concentration of α amanitin. Studies *in vitro* (ref. 3 and D.S. *et al.*, manuscript in preparation) indicate that Ama102 cells possess an RNA polymerase II which retains about 30% of its activity in 0.1 $\mu\text{g ml}^{-1}$ α amanitin, a concentration sufficient to inactivate completely the Ama⁺ polymerase II. If α amanitin has a similar inhibitory action *in vivo* and *in vitro*, the normal growth of Ama102 cells in α amanitin coupled with the partial resistance of its RNA polymerase II activity to α amanitin *in vitro* seems paradoxical. Does the mutant Ama102 cell grow normally in a α amanitin with only 30% of its normal RNA polymerase II active, or can the RNA polymerase II activity increase when the mutant is grown in α amanitin? The experiments reported here indicate that Ama102 cells can compensate for the partial inactivation of RNA polymerase II by α amanitin by increasing the RNA polymerase II activity.

The activity profile of the RNA polymerases obtained by DEAE-Sephadex chromatography of extracts of Ama102 cells grown for many generations in the absence of α amanitin

(Fig. 1a) shows that polymerase II is partially resistant to 0.1 $\mu\text{g ml}^{-1}$ α amanitin. When these cells were grown for 4 d in the presence of α amanitin (3 $\mu\text{g ml}^{-1}$) the polymerase II activity was completely resistant, representing a threefold increase in resistant activity (Fig. 1b). There seemed to be no corresponding increase in polymerase I activity. The Ama102 cells responded to the exposure to α amanitin by modulating the activity of resistant polymerase II. This increased level of α amanitin-resistant RNA polymerase II activity was maintained even after 30 d of continuous growth in α amanitin (data not shown).

The α amanitin sensitivity of RNA polymerase I and II purified by DEAE-Sephadex chromatography from Ama⁺ and Ama102 cells grown with and without α amanitin was characterised further (Fig. 2). RNA polymerase I from both wild type and mutant cells was not inhibited by up to 50 $\mu\text{g ml}^{-1}$ α amanitin, while the Ama⁺ RNA polymerase II was inhibited completely by 0.1 $\mu\text{g ml}^{-1}$. About 30% of the RNA polymerase II from Ama102 cells grown in the absence of α amanitin and all of it from Ama102 cells grown in 3 $\mu\text{g ml}^{-1}$ α amanitin was resistant to 0.1 $\mu\text{g ml}^{-1}$. This α amanitin-resistant RNA polymerase II activity was only inhibited by much larger concentrations of α amanitin (50% inhibition with 5 $\mu\text{g ml}^{-1}$). This α amanitin-resistant activity is distinguished from RNA

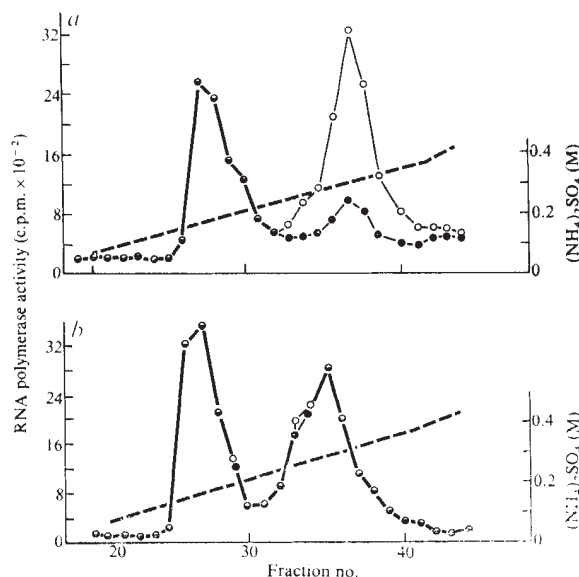


Fig. 1 DEAE-Sephadex chromatography of RNA polymerase activities in α amanitin-resistant rat myoblast Ama102 grown in the absence and presence of α amanitin. Ama102 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% foetal calf serum (Gibco) at 37 °C under 100% humidity in 5% CO₂-95% air. 10⁸ cells, washed once with citrate saline, were resuspended in TGMED (50 mM Tris-HCl (pH 7.9 at 22 °C), 25% (v/v) glycerol, 5 mM MgCl₂, 0.1 mM EDTA and 1 mM dithiothreitol) containing 10⁻⁴ M phenylmethylsulphonylfluoride. Ammonium sulphate (4 M, pH 7.9) was added to a final concentration of 0.3 M, and the suspension was sonicated, diluted with 2 volumes of TGMED and centrifuged (30 min, 4 °C, 125,000g). The supernatant was diluted with TGMED to 0.03 M in ammonium sulphate and used for chromatography on DEAE-Sephadex A-25 (0.9 × 12 cm), eluting with 10 ml of TGMED, 0.03 M in ammonium sulphate, then a 40 ml linear gradient, 0.03-0.5 M ammonium sulphate in TGMED. RNA polymerase activity was determined in column fractions (1 ml) by addition of 40 μl of enzyme solution to 80 μl of assay mixture⁹ which contained 2 μCi ³H-UTP (18-22 Ci mmol⁻¹). The reactions, 20 min at 30 °C, were terminated by adding 0.01 ml 5% (w/v) sodium dodecylsulphate containing 0.25 M sodium pyrophosphate. 100 μl of the reaction mixture was spotted on 2.4 cm Whatman DE-81 filter disks and the filters were washed and counted². a, Cells grown in the absence of α amanitin; b, cells grown for 96 h in the presence of α amanitin (3 $\mu\text{g ml}^{-1}$). RNA polymerase activity was assayed in the absence of (○) and presence of (●) α amanitin (0.1 $\mu\text{g ml}^{-1}$).

polymerase III both by its elution from DEAE-Sephadex before the small amounts of polymerase III detected (Fig. 1) and by its inhibition by concentrations of α amanitin lower than those reported to inhibit polymerase III⁵.

We have examined the time course of the increase in α amanitin-resistant RNA polymerase II activity in Ama102 cells (Fig. 3). Cells grown in the absence of drug for many generations were put into α amanitin ($3 \mu\text{g ml}^{-1}$) and the total

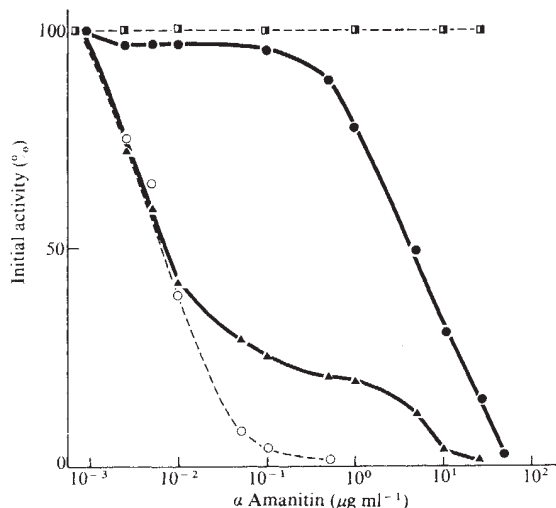


Fig. 2 α Amanitin sensitivity of RNA polymerase activities from Ama102 and Ama⁺ cells purified on DEAE-Sephadex. Assay conditions were as described in the legend to Fig. 1 except that each assay contained $5 \mu\text{Ci } ^3\text{H-UTP}$. $\square$, RNA polymerase I from Ama⁺; $\blacksquare$, RNA polymerase I from Ama102 grown for 96 h in α amanitin ($3 \mu\text{g ml}^{-1}$); $\circ$, RNA polymerase II from Ama⁺; $\blacktriangle$, RNA polymerase II from Ama102 cells grown in the absence of α amanitin; $\bullet$, RNA polymerase II from Ama102 cells grown for 96 h in α amanitin ($3 \mu\text{g ml}^{-1}$).

and α amanitin-resistant RNA polymerase activities in cell lysates were determined at various times under assay conditions where RNA polymerases I and III together accounted for no more than 17% of the total activity measured (D.S. *et al.*, unpublished). Within the first 8 h there was a decline in the total specific activity (per mg total protein) of the RNA polymerase, probably resulting from the entry of α amanitin into the cells with consequent inhibition of RNA polymerase II activity. Within 16 h, however, the total RNA polymerase specific activity had increased to approximately its original value. This was entirely accounted for by an increase in α amanitin-resistant RNA polymerase activity. After 48 h of growth in α amanitin only α amanitin-resistant polymerase activity was detected in cell lysates. Removal of the α amanitin at 48 h led to a progressive decrease in the specific activity of α amanitin-resistant polymerase, the total RNA polymerase activity remaining constant. Fifty-six hours after removal of α amanitin, the levels of total and α amanitin-resistant RNA polymerase had returned to the prechallenge levels. The rapid change in the relative level of α amanitin-resistant RNA polymerase argues against the trivial possibility that these changes were due to selection of Ama^r and Ama^s subpopulations of cells within the Ama102 culture. Similar phenotypic regulation of RNA polymerase II activity has been observed in our laboratory in several other clones of independently isolated Ama^r myoblast mutants (M. Crerar, S. Andrews, and D.S., unpublished).

These data indicate that RNA polymerase II activity in Ama102 is subject to regulation which is independent of that of the activity of RNA polymerase I. We suggest that RNA polymerase II synthesis in rat myoblasts is regulated autogenously as RNA polymerase synthesis seems to be in bacteria⁶. Based on experiments using *E. coli rif^r/rif^s* merodiploids⁷ and λrif infected *Escherichia coli*⁸ it has already been suggested

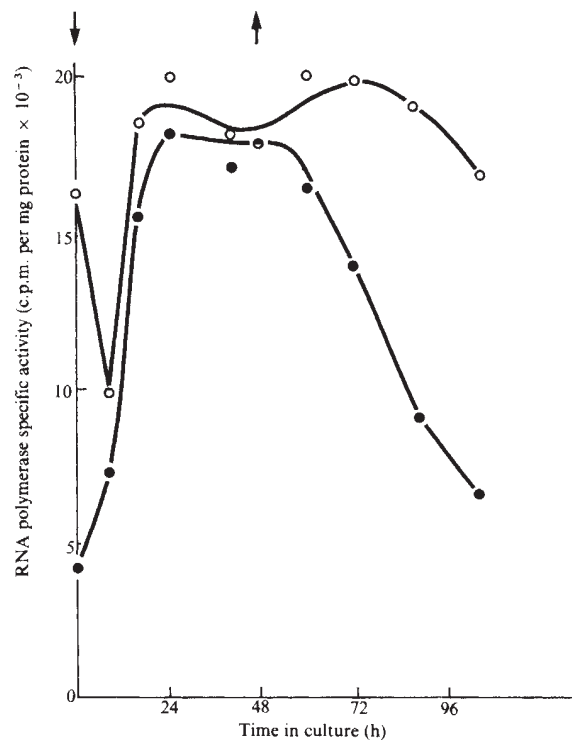


Fig. 3 Changes with time of the RNA polymerase-specific activities in cell lysates of Ama102 cells grown in the presence and after removal of α amanitin ($3 \mu\text{g ml}^{-1}$). Ama102 cells, seeded in Falcon flasks (75 cm^2) at different densities to yield approximately 3×10^6 cells per flask when collected, were grown in the drug for various times up to 48 h. α Amanitin was removed from other flasks after 48 h; the cells were washed and fresh medium minus the drug was added. Ama102 cells were collected again at various times after removal of drug. The specific activities of RNA polymerase in the presence and absence of α amanitin ($0.1 \mu\text{g ml}^{-1}$) were determined in the cell lysates (3×10^6 cells suspended in 0.5 ml TGMED) prepared by four cycles of freezing and thawing (using liquid nitrogen and a 30°C water bath). The assay conditions were as described in Fig. 1, except that each assay was made 0.4 M in ammonium sulphate to enhance the relative activity of RNA polymerase II compared with RNA polymerase I (D.S. *et al.*, manuscript in preparation). Total protein was determined in these lysates by the method of Lowry *et al.*¹⁰. The doubling time of Ama102 cells was 15 h in either the presence or absence of $3 \mu\text{g ml}^{-1}$ α amanitin. $\circ$, Total RNA polymerase activity; $\bullet$, α amanitin-resistant RNA polymerase activity. Downward arrow, addition of α amanitin; upward arrow, removal of α amanitin in the culture media.

that RNA polymerase may act as its own repressor. Our results suggest that this mode of gene regulation may extend to eukaryotes as well, although other mechanisms such as enzyme modification and end product feedback inhibition may regulate RNA polymerase II activity. A first requirement of this hypothesis of autogenous regulation will be the correlation of the increase in α amanitin-resistant polymerase II activity with an increase in *de novo* enzyme synthesis.

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DONALD G. SOMERS
MARK L. PEARSON

Department of Medical Genetics,
University of Toronto,
Toronto, Canada M5S 1A8

C. J. INGLES

Banting and Best Department of
Medical Research,
University of Toronto,
Toronto, Canada M5G 1L6

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A blocked structure at the 5' terminus of mRNA from cytoplasmic polyhedrosis virus

METHYLATION of a specific nucleotide occurs at the initial stage of transcription of the double-stranded RNA genome in cytoplasmic polyhedrosis virus (CPV) in the presence of the methyl-group donor, S-adenosyl methionine (SAM)¹. Methylation of mRNA has been reported recently not only for other viruses^{2,3} but also for a mouse L-cell⁴. Since methylation seems to be coupled with the initiation of mRNA synthesis in CPV, the structure of the initiation (5') terminus of mRNA transcribed from CPV by the virus-containing RNA polymerase is of particular interest.

In the absence of SAM, single-stranded RNA transcribed from CPV *in vitro* has the terminal structure ppA-G-Y-. All ten kinds of mRNA, which correspond to the ten genome segments, have the same terminal structure⁵. The mRNA has the same sequence as the 5'-terminal-modified strand of the double-stranded RNA genome in CPV⁷ as well as in reovirus⁸. When SAM is added to the *in vitro* reaction mixture of CPV transcription, RNA synthesis is greatly stimulated⁷. The RNA synthesised in the presence of SAM is the same size as the RNA transcribed in the absence of SAM, but the former is methylated in the ribose moiety (probably the 2'-position) of an adenylic acid residue in the 5'-terminal region¹. We report the structural analysis of the 5'-terminal part of the RNA synthesised in the presence of SAM, showing that the 5'-terminal adenylic acid residue is methylated in the ribose moiety and, further, that the 5'-terminal phosphates are blocked by 7-methylguanylic acid.

Isolation of CPV and its RNA^{9,10}, construction of the system for *in vitro* RNA synthesis in the presence of SAM¹, and analytical methods for the oligonucleotides^{6,7}, have been published previously.

The mRNA synthesised from CPV in the absence of SAM carries α,β -diphosphates at the 5'-terminal adenosine⁶. Also in the presence of SAM, β -³²P-labelled ATP is incorporated into the newly synthesised mRNA. CPV mRNA doubly labelled with β -³²P-ATP and ³H-methyl SAM was prepared. When this was digested by various ribonucleases or alkali, both the radioactivities of ³²P and ³H were always found in one component in the DEAE-cellulose-urea column chromatography (details will be published elsewhere). This means that methylation occurs only at the 5'-terminus. These experiments suggest that the 5'-terminal sequence is ppAm-G-Y- (see Fig. 3) was not, however, changed by the phosphomonoesterase treatment (Fig. 1a). Therefore, phosphate groups at the 5'-terminal nucleotide must be blocked by some unknown material, X, as XppAm.

This 5'-terminal material (XppAm) was then treated with venom phosphodiesterase. The products were separated by paper electrophoresis. As shown in Fig. 1b five components were detected by radioactivity, one of which (III) was non-degraded original material. Component II was identified as pAm (2'-O-methyladenosine-5'-phosphate). This contains a ³H-methyl group and moves with standard pAm, synthesised chemically¹¹, on paper chromatography. When this was digested with phosphomonoesterase, ³H-radioactivity was detected in 2'-O-methyladenosine. Component V is inorganic phosphate

carrying radioactivity (³²P). Component I at the origin (charge zero) was considered to be phosphorylated material X (Xp), because phosphomonoesterase treatment for this gave a material X, which moves to the cathode faster than any normal nucleoside at pH 3.5, indicating that it has a positive charge. From other experiments (details will be published elsewhere) the material X is labile in alkali, and contains a *cis*-diol. As candidates for X, SAM and 7-methylguanosine (m⁷G) were chromatographed and electrophoresed with ³H-methyl-X. In each case the radioactivity of ³H-X was simply superimposed on the ultraviolet-absorbing spot of 7-methylguanosine (examples are shown in Fig. 2). Therefore, X must be 7-methylguanosine.

In component I, the plus charge of X seems to be cancelled by the minus charge of a phosphate group at pH 3.5. As this phosphate did not carry radioactivity, this is not the β -³²P of the terminal 2'-O-methyladenylic acid. When α -³²P-labelled

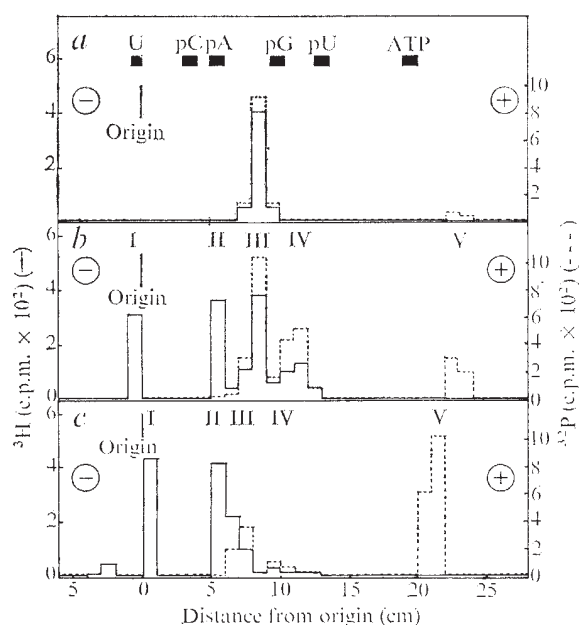


Fig. 1 Paper electrophoresis of the terminal oligonucleotide and its phosphodiesterase digest. *a*, Oligonucleotides obtained by *Penicillium* nuclease and phosphomonoesterase. CPV mRNA solution (100 μ l; ³H: 60,000 c.p.m.; ³²P: 60,000 c.p.m.) was mixed with 5 μ l 0.1 M sodium acetate buffer (pH 6.0) and 20 μ l *Penicillium* nuclease solution, and incubated at 37°C for 90 min. The digest was spotted on Whatman 3MM paper and electrophoresed in pH 3.5 buffer (5% acetic acid, pH adjusted with morpholine), containing 5 mM EDTA, at 40 V cm⁻¹ for 50 min in cooled hexane. After drying in a gentle air stream, the paper was cut into pieces (1 cm wide) and the radioactivity in each piece counted in 5 ml PPO- and POPOP-containing toluene.

When the sample (20 μ l) was further mixed with 1 μ l phosphomonoesterase solution after adjusting pH to 8.0 with 1.0 M Tris-HCl buffer (pH 8.0) and incubated at 37°C for 30 min, a similar electrophoretogram was obtained. This means that the phosphate groups in the sample oligonucleotide are protected from phosphomonoesterase.

b, Phosphodiesterase digest of *a*. The radioactive region in an electrophoretogram shown in *a* was extracted with 150 μ l distilled water. This sample (75 μ l) was mixed with 10 μ l 0.5 M phosphate buffer (pH 7.2), 1 μ l 1 M MgCl₂, 2 μ l venom phosphodiesterase (Worthington; 7.5 mg ml⁻¹), and distilled water to total 200 μ l. The mixture was incubated at 37°C for 7 min. Paper electrophoresis as in *a*.

c, Stronger digestion of *a* with phosphodiesterase. The same sample as the starting material in *b* was mixed with 20 μ l venom phosphodiesterase (15 times the concentration of *b*) and buffer containing MgCl₂ (same concentration as *b*) in 130 μ l total. The mixture was incubated at 37°C for 30 min. Paper electrophoresis was carried out at 36.4 V cm⁻¹ for 50 min, otherwise as in *a*.

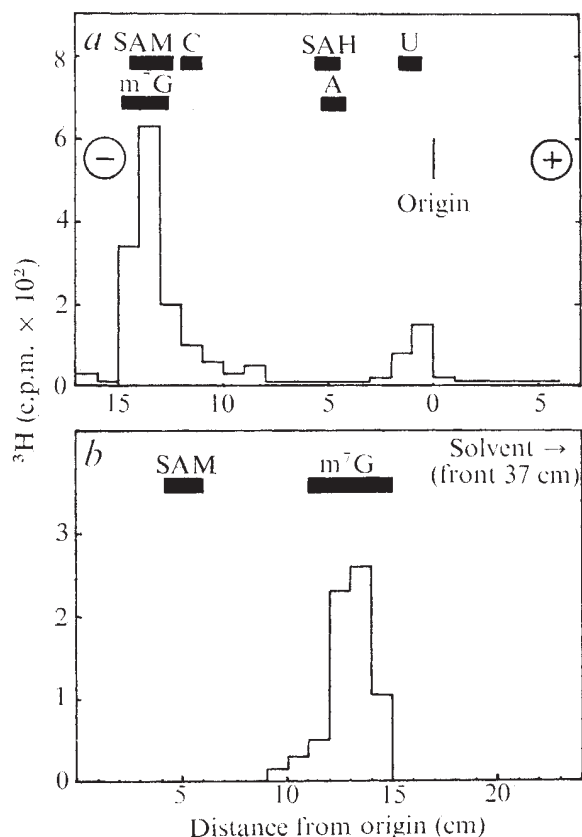


Fig. 2 Identification of 7-methylguanosine linked to the terminal part of CPV mRNA. Fraction I in the phosphodiesterase digest of the terminal part of CPV mRNA, obtained from the *Penicillium* nuclease digest of the labelled mRNA (b), was eluted with distilled water, and digested with phosphomonoesterase under the same conditions as in Fig. 1. a. The digest was spotted on Whatman 3MM paper and electrophoresed at 50 V cm^{-1} for 40 min in the same buffer as used in Fig. 1. b. The digest was spotted on Whatman 3MM paper and developed with *n*-butanol-acetic acid- H_2O (5:1:4, V/V/V). As reference materials, SAM, S-adenosylhomocysteine (SAH), 7-methylguanosine, adenosine, cytidine, uridine, and the mixture of 5'-nucleotides (latter not shown) were run with the sample. Counting as in Fig. 1.

guanosine-5'-triphosphate was added as a substrate, ^{32}P was incorporated into this component I. Therefore, component I is 7-methylguanosine-5'-phosphate (pm^7G). Component IV was considered to be the material X combined with two phosphate groups. By phosphomonoesterase treatment, ^{32}P was released as inorganic phosphate, together with ^3H -methyl-X. Thus component IV must be component I (Xp) further phosphorylated with ^{32}P phosphate which was incorporated from β - ^{32}P -ATP; that is, IV must be Xp^{32}P . Two phosphate groups would yield a minus charge for IV. Components III and IV in Fig. 1b seem to remain because of incomplete hydrolysis by phosphodiesterase, as these components diminish when stronger conditions for hydrolysis are applied (Fig. 1c). In the latter case there are three major components: I, pm^7G - ^3H -methyl; II, pAm - ^3H -methyl; and V, p^{32}P -phosphate.

Since the phosphate groups are blocked and protected from the phosphomonoesterase attack, 7-methylguanosine-5'-phosphate must be linked to the β -phosphate of ppAm through a pyrophosphate linkage. In fact pyrophosphatase treatment on the starting material yields 7-methylguanosine-5'-phosphate. Therefore, the starting 5'-terminal material has the structure $\text{m}^7\text{Gp}^5\text{pp}^5\text{Am}$ as shown in Fig. 3. The number of phosphates between X and Am must be three, as the plus charge in m^7G was cancelled with one negative charge from phosphates.

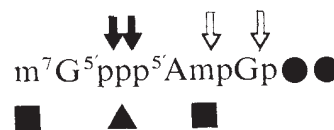


Fig. 3 A proposed structure of the 5' terminal part of CPV mRNA and the enzyme-attacking sites. Radioactive components: (■, ^3H -methyl; ▲, ^{32}P). White arrow, attacking of *Penicillium* nuclease; black arrow, attacking of venom phosphodiesterase (after *Penicillium* nuclease digestion).

This consideration explains clearly the behaviour of the 5'-terminal material in DEAE-cellulose-urea column chromatography and in paper electrophoresis. Recently a similar blocked structure was reported for the 5'-terminus of low molecular weight nuclear RNAs from Novikoff hepatoma ascites cells¹³.

According to this analysis of the terminal nucleotide of CPV mRNA synthesised in the presence of SAM, the methylation occurs exactly at the initiation point (5'-terminus) of mRNA, as one of the genome duplex strands (ref. 7 and K.M. and Y.F., unpublished). In addition to the methylation of the ribose moiety of the terminal adenosine, 7-methylguanylic acid combines with the 5'-terminus of RNA by the 5'-5' pyrophosphate linkage. These modifications at the 5'-terminus of mRNA could play an important role in the initiation of mRNA synthesis and/or the function of mRNA. The blocked structure as well as the methylated 5'-terminal nucleotide of mRNA found here would be resistant to exonucleolytic attack, lengthening the life of mRNA. Alternatively, this modified structure may be necessary for completion of transcription, transportation and/or translation by forming a specific tertiary structure. Although the biological meaning of this modification of mRNA structure is not clear now, a similar modification has also been found in mRNA of vaccinia virus containing DNA as its genome¹⁴.

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YASUHIRO FURUICHI
KIN-ICHIRO MIURA

National Institute of Genetics
Mishima, 411, Japan

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Different effector cell types in antibody-dependent cell-mediated cytotoxicity

ANTIBODY-DEPENDENT cell-mediated cytotoxicity has been demonstrated *in vitro* using a number of continuously cultivated cell lines^{1,2} and nucleated erythrocytes³ as targets. Although the biological role of this cytotoxicity is not yet clear, it represents a potentially efficient cytotoxic mechanism and considerable interest has been shown in the characterisation of the effector cells.

The use of antibody-coated chicken erythrocytes (E-Ab) as targets has technical advantages and they have been widely used in assaying antibody-dependent effector cells. The report that effector cells in mouse spleen are monocyte-like⁴⁻⁶ was not easily reconciled with observations in our laboratory and elsewhere using cell line cells as targets. These authors assumed that the effector cells in mouse spleen active against E-Ab are the same as the effectors active against antibody coated cell line targets. It has been suggested that polymorphonuclear cells may participate in the reaction but even when these cells have been eliminated the possibility remains that more than one effector is involved. We have, therefore, made a direct comparison of the cells killing antibody-coated erythrocytes with those killing cell line cells coated with homologous antibody.

The results show that a population of effector cells highly active against antibody-coated erythrocytes has little or no activity against antibody-coated cell line cells.

The cytotoxicity assay towards antibody-coated chicken erythrocytes (E-Ab) was performed essentially as described by others^{3,6}. In addition two cell lines have been studied; the mouse mastocytoma P815 (M) and the human FL cell line (F)⁷. Each target was tested in the presence and absence of the appropriate antiserum at a dilution of 1:1,000; rabbit anti-E, rat anti-M and rat anti-human

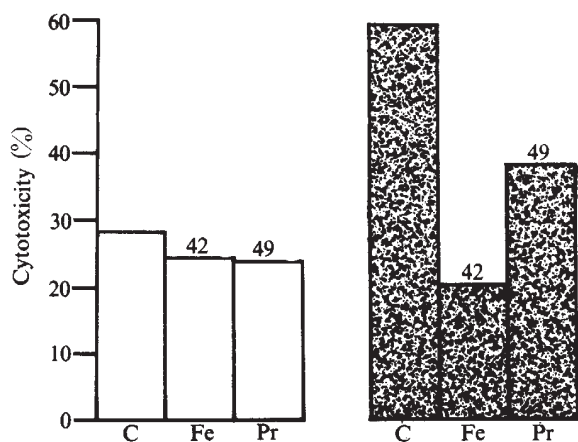


Fig. 1 Treatment of spleen cells with carbonyl iron. Protein coating was carried out by suspending 200 mg sonicated carbonyl iron in 10 ml of medium containing 10% foetal calf serum and incubating on a roller at 37°C for 60 min. Uncoated particles were treated in serum free medium. 100 mg of iron was added to 5×10^7 cells in 1 ml in a tube and incubated at 37°C for 30 min. The unadhered cells were removed with the aid of a magnet and the iron particles resuspended in medium. These washings were recovered in the same way, pooled with the first cell suspension and adjusted to 5×10^6 ml⁻¹. C, Untreated control; Fe, uncoated iron particles; Pr, protein-coated iron particles. The percentage of cells recovered relative to the controls is shown at the top of the histograms. Clear blocks are M-Ab cytotoxicity; hatched blocks are E-Ab cytotoxicity.

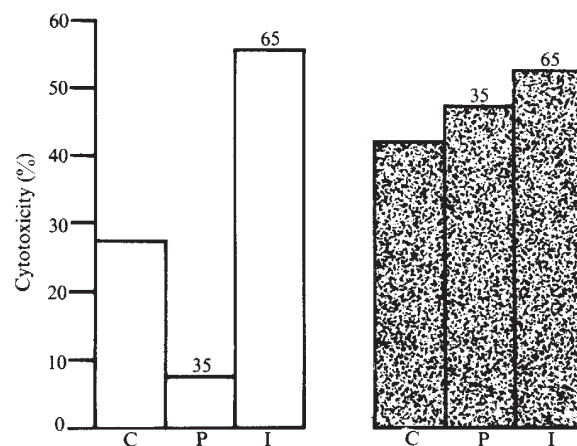


Fig. 2 Fractionation on Ficoll-Trisil. 5 ml of cell suspension was layered over 10 ml of Ficoll-Trisil and centrifuged for 10 minutes at 500g. Cells from the interface (I) and the pellet (P) were washed and suspended at 5×10^6 ml⁻¹. The percentage of total recovered cells in the pellet and interface is indicated at the top of the histograms. Total cell recovery was 80%. Clear blocks are M-Ab cytotoxicity; hatched block are E-Ab cytotoxicity.

erythrocyte. Except where stated the concentration of target cells was 10^5 ml⁻¹ and of effectors 5×10^6 ml⁻¹; the percentage ⁵¹Cr release was determined after mixing equal volumes of effectors and target cell suspensions and incubating for 4 h at 37°C. The percentage of cytotoxicity was calculated as: (test—control)/(maximum—control) using as control the isotope release in the absence of antibody and the values for maximum release obtained by lysis of E in water (approximately 65%) and twice freezing and thawing the cell lines in hypotonic solution (approximately 80%). The data have been analysed by a multi-variable analysis of variance using logarithmic transformation of percentage ⁵¹Cr release. All the values presented as cytotoxicity represent killing significant at the 1% level.

In all the experiments the same preparation of effector cells was tested on the different targets under identical conditions. The results with the two cell lines were similar in each case except that the mastocytoma was more susceptible to killing.

In our hands mouse spleen cells show high activity against E-Ab but only low activity against antibody coated cell line cells. For example, BALB/c spleen cells tested at a ratio of 50:1 for 4 h on E-Ab, M-Ab and F-Ab gave 47%, 3% and no detectable cytotoxicity respectively, whereas under the same conditions Agus rat spleen cells gave 48%, 27% and 10% respectively. This conclusion is supported by other work (for example ref. 8) using antibody coated cell line cells and mouse spleen effectors where high ratios and a long incubation period (100:1, 16 h) were necessary for cytotoxicity. Other experiments (C. J. S., to be published) established that the difference between the two species was not the result of the source of antibody as similar results were obtained with mouse antibody. Because of this relative deficiency in mouse spleen we have used Agus rat spleen effectors in these experiments.

Treatment of spleen cells with carbonyl iron considerably reduces the activity towards E-Ab while only slightly reducing and sometimes enhancing the activity towards M-Ab. The cell recoveries suggested that this technique

was removing more than just the phagocytic cells. In an attempt to differentiate between phagocytosis and adherence the iron particles were first coated with protein. Examination under the microscope indicated that the coated particles were readily phagocytosed. Figure 1 shows an experiment in which uncoated and protein-coated iron particles were used. Uncoated iron shows a differential removal of activity towards E-Ab whereas protein-coated iron shows very little differential removal of activity towards E-Ab. The reason for this is not entirely clear; protein coated particles consistently remove fewer cells than uncoated particles, although coated particles seem to be readily phagocytosed. This suggests that these effector cells are being removed by adherence rather than phagocytosis. In an experiment using BALB/c spleen effectors, activity against E-Ab was decreased from 47% to 7% by uncoated carbonyl iron, while the activity towards M-Ab remained unchanged (3.5% to 3.3%). Thus, in both species, the activity towards E-Ab is more adherent than the activity towards M-Ab.

Figure 2 shows the distribution of activity after centrifugation on Ficoll-Triosil interface. Under these condi-

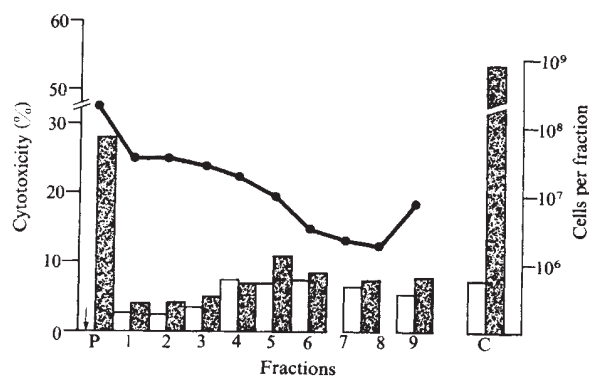


Fig. 3 Fractionation on BSA gradient (modified from 10) 3.6×10^8 spleen cells were suspended in 1 ml of 35% BSA and placed in the bottom of a 10-ml centrifuge tube. A linear gradient from 35% to 20% BSA was formed over the cells. The tube was centrifuged in a swing out head (MSE Superspeed 65) at 20,000g for 30 min at 20°C. Approximately 1 ml fractions were collected from the bottom after puncturing the tube. The pellet was recovered with a Pasteur pipette. Cells were washed twice in serum free medium before counting and resuspending in medium at 5×10^6 ml⁻¹. Cytotoxicity was determined after 5 h incubation. Cytotoxicity towards E-Ab is shown hatched, cytotoxicity towards F-Ab as clear blocks. Total cell recovery was 65% and the distribution is shown (●). Fractions 7 and 8 were pooled for the cytotoxicity assay. Unfractionated spleen control (C). The pellet (P) showed no activity against either cell line (arrow) but strong activity against E-Ab.

tions the cells recovered from the pellet are active against E-Ab with relatively little activity against M-Ab. The cells at the interface retain both activities.

In an attempt to make a better preparation of the lower density cells centrifugation was carried out at 1,000g. The cells recovered from the interface were then further purified by treatment with carbonyl iron. These cells gave 26.5% cytotoxicity with M-Ab and 7.5% with E-Ab.

These results indicate that the high density cells (pellet) active against E-Ab have little activity against M-Ab whereas the lower density cells with activity against M-Ab are less active against E-Ab. In each case it is not clear whether the residual activity against the other target cell is due to incomplete separation of the two types of effector.

In a further effort to improve the separation of the two types of effector cells, we have investigated isopycnic centrifugation on different ranges of continuous bovine

serum albumin (BSA) gradients. Using a gradient from 35–20% BSA the cells recovered from the pellet show strong activity towards E-Ab with no detectable activity towards M-Ab or F-Ab. On the other hand, there is a population of cells with a peak at the middle of the gradient which is active towards all targets. Strong background killing of M in the absence of antibody was, however, also found in these fractions. The reasons for this are unclear (it may be caused by immune complexes in the BSA) but it made the calculations of cytotoxicity unreasonable and so only the data obtained with F-Ab are presented (Fig. 3).

As the two cell lines we have used are quite different in origin and susceptibility to killing, it seems reasonable that other susceptible cell lines will be killed by the same population of effector cells. But the data obtained with E-Ab, at least with rodent spleen effectors, must be interpreted with caution and cannot necessarily be generalised to cell line targets.

Greenberg *et al.* implied that treatment of mouse spleen with iron removed only cells with a phagocytic function, leaving non-phagocytic but adherent effector cells ("null cells"). It stems from our data and those of Dennert and Lennox¹¹ that the adherent properties of these cells can render them susceptible to almost total removal by iron, along with actively phagocytic cells. This effect may be responsible for the disagreement in the conclusions of various authors using E-Ab target cells^{6,11,12}.

In conclusion, rodent spleen contains two populations of antibody-dependent cytotoxic cells. One is a low density, non-adherent cell with activity towards cell line targets and chick erythrocytes. Mouse spleen is a relatively poor source of these cells compared with rat spleen. The second type of cell is a higher density, adherent cell. This is clearly the cell characterised by Greenberg *et al.* as "null cells". These cells are present to a similar extent in both rat and mouse spleen, they are cytotoxic towards chick erythrocytes but not cell line targets.

We have avoided the suggested terminology (K-cells)¹³ because it is not clear whether it should be used to include both types of effector cell. We wish to thank Drs A. C. Allison and E. M. Lance for criticising the manuscript.

C. J. SANDERSON
I. A. CLARK
G. A. TAYLOR

Clinical Research Centre,
Watford Road,
Harrow HA1 3UJ, UK

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Age limitation of perceptual span

DIFFERENCES in speed of perception and performance between younger and older human adults sometimes increase proportionately with task difficulty, but sometimes remain constant. Gregory¹ has argued that the added time is adaptive and has shown how a constant component, such as age-associated

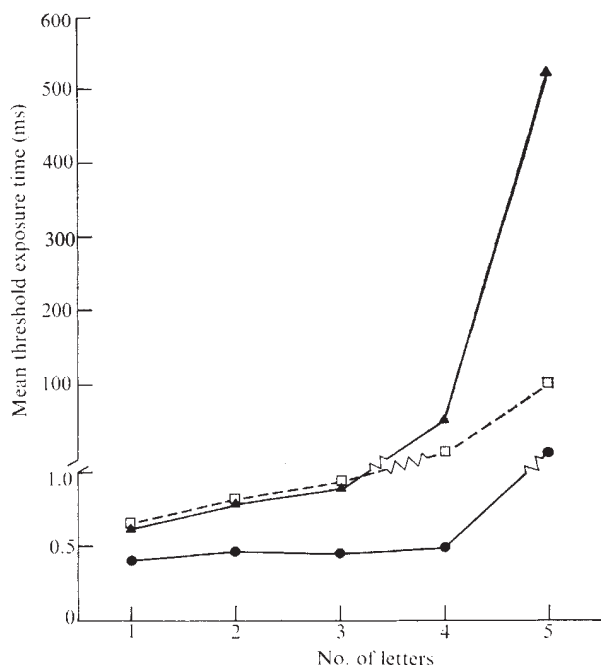


Fig. 1 Absolute thresholds of time exposure for correct identification of varying numbers of letters by different age groups. ●, 20-30 age group; □, 40-50 age group; ▲, 60-70 age group.

rise in 'noise' level, could result in a linear increase of decision time. Welford² suggests that the constant age effect occurs when signals are brief, whereas when perception is not limited, proportionate increases are manifested. We report here an example of a constant difference between age groups in absolute time thresholds followed by an abrupt change in a situation where signals are brief. The implication seems to be that two different deficiencies are operating, both of which could perhaps ultimately be attributed to 'noise'. Participants in the experiment were required to identify varying numbers of letters and the sudden increase in required time indicates a reduction in the visual perceptual span of older people.

A modified staircase method³ was used to determine the absolute time thresholds for correct reporting of 1, 2, 3, 4 and 5 upper case consonants. In estimating thresholds for more than 1 letter, correct order of report was required. Black stimuli (Letraset 719, 60-point spaced 10 mm centre to centre) were printed in the middle of a white card and displayed binocularly in a 3-channel tachistoscope with a dark pre- and post-exposure field. After every successful or unsuccessful trial the stimulus was replaced by a member of a set of 20 at each level of difficulty. Five male and five female volunteers in each of the age ranges 20-30, 40-50 and 60-70 were tested. To equate for age differences⁴, subjects in the young, middle and old age groups were dark adapted for 5, 7 and 10 min respectively, the experimental room having very low illumination (one 25-W red light bulb). Calculations are based on the mean of two estimates of threshold for each subject, order of presentation being 2, 3, 1, 4, 5, 3, 2, 4, 1 and 5 letters.

Results are given in Fig. 1 and show a dramatic increase in the exposure time required by the oldest group to identify 5 letters. Among that group there was only one exception to the very large time increment. Two members of the middle age group demonstrated a comparable increase, and there was one case in the oldest group with a similar increase at 4 letters. The youngest group had significantly lower thresholds than the two older groups for 1, 2 and 3 letters as well as a lower threshold than the oldest for 5 letters. The difference

between the middle and oldest group was only significant for 5 letters.

When stimuli were below threshold all age groups made most of their errors in the middle letters of a series. It is therefore unlikely that the limited perceptual span of the elderly is the result of poorer peripheral vision. For the same reason, output interference is contraindicated as the primary explanation, since such interference should lead to more frequent errors in the report of the last letter of a series. Nevertheless, a sensory memory storage deficit could be a major component in the reduction of perceptual span with age. This possibility is not excluded by the equivocal results of Abel's study⁵ using Sperling's⁶ partial report method, where exposure times of one half second were used. Many older participants in the present experiment made statements implying that they had seen a letter, but that it had disappeared before it could be identified. Similar comments have been reported from younger subjects in previous investigations⁷ and were made by members of younger age groups in a study we ran on time thresholds for more than five letters. The sudden increase in time we found with 5 letters among older people occurred at 6 or 7 letters in the young. Here, too, sensory storage is likely to be a major factor in limiting perceptual spans. Indeed, it would not be wrong to describe the perceptual span as a sensory memory span, since readout follows the disappearance of the stimulus and therefore must be formed from a lingering image.

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DAVID SCHONFIELD
LARRY WENGER

Psychology Department,
University of Calgary,
Calgary, Alberta, Canada

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Errata

In the letter "Inhibition of adenyl cyclase by an exotoxin of *Bacillus thuringiensis*" by D. G. Grahame-Smith, P. Isaac, D. J. Heal and R. P. M. Bond (*Nature*, **253**, 58; 1975) Figs 1 and 2 were transposed. The legends are correct as they stand.

In the letter "Sustained oscillations of acetylcholine during nerve stimulation" by Y. Dunant, P. Jirouneck, M. Israël, B. Lesbats and R. Manaranche (*Nature*, **252**, 485; 1974) the label on the abscissa of Fig. 1a should read 'Time (s)' and not as printed.

In the article "New observations of the angular diameter-redshift relation for radio sources" by A. Hewish, A. C. S. Redhead and P. J. Duffett-Smith (*Nature*, **252**, 657; 1974) there is a misprint on page 659. In line 18, $0.17'' \pm 0.3''$ should read $0.17'' \pm 0.03''$.

In the article "Two types of resistance to polyene antibiotics in *Candida albicans*" by C. C. HsuChen and D. S. Feingold (*Nature*, **251**, 656; 1974), the following corrections should be made to the legend of Table 2. In line 2, for CH_3COOH read MeOH ; the expression in lines 4 and 5 should read $[(\text{c.p.m. of given phospholipid})/(\text{total c.p.m. in phospholipid fraction})] \times 100$, and not as printed.

matters arising

Simplification of palindromic telomere theory

CAVALIER-SMITH's motive in devising his palindromic model for telomeres¹ was to surmount the obstacle of the replication of the 3' end of a linear molecule of DNA (Fig. 1). His palindromic end with related hairpin bend, however, makes the 3' end unnecessary and therefore, by the usual rules of natural selection, improbable.

This can be achieved by a slight modification of his original model (Fig. 1). This has the attraction of not only being simpler, five steps replacing seven, but also of explaining the special property of telomeres which distinguishes them from any other end of a linear DNA molecule, namely a chromosome break: their stability.

The essence of my model is that the self-paired telomeric palindrome is the normal condition, in G₁, G₂ and M. Then the phosphate backbone of the double helix is continuous through the telomere. Similarly, DNA replication

will proceed continuously through the telomere thus making the terminal RNA primer redundant.

This self-paired state is temporarily lost as the result of S and restored via denaturation. The endonuclease that nicks the end of the palindrome and allows the unfolding and refolding in the new configuration must be telomere specific, making it probable that all telomeres of one genome carry the same palindrome. Without this manoeuvre sister chromatids would remain attached end-to-end leading to non-disjunction at anaphase.

A philosophical attraction of my modification of the model is that the sister molecules (sister chromatids) participate equally in the process, in contrast with the original model in which one complete molecule is produced at once and the sister molecule is incomplete and has to go through a complex manoeuvre to correct itself. My model also requires one S phase, at the start. The original model starts and ends with DNA synthesis.

Finally, my model may be open to experimental confirmation. If the telomeric palindrome is long enough, the asymmetry between sister telomeres, one being old and one wholly new, might be detectable in autoradiographs of the first metaphase after labelling in S with ³H-thymidine. Wilson and Thomas report palindromes up to 12,000 nucleotides long². As there are only two telomeres per chromosome the possibility of recognising telomeric palindromes in DNA extracts would be like searching for the proverbial needle (or should we say hairpin?) in a haystack.

This work was supported by the Cancer Research Campaign and the Medical Research Council.

A. J. BATEMAN

Paterson Laboratories,
University Hospital of South Manchester,
Manchester M2D 9BX, UK

¹ Cavalier-Smith, T., *Nature*, **250**, 467-470 (1974).

² Wilson, D. A., and Thomas, C. A., *J. molec. Biol.*, **84**, 115-144 (1974).

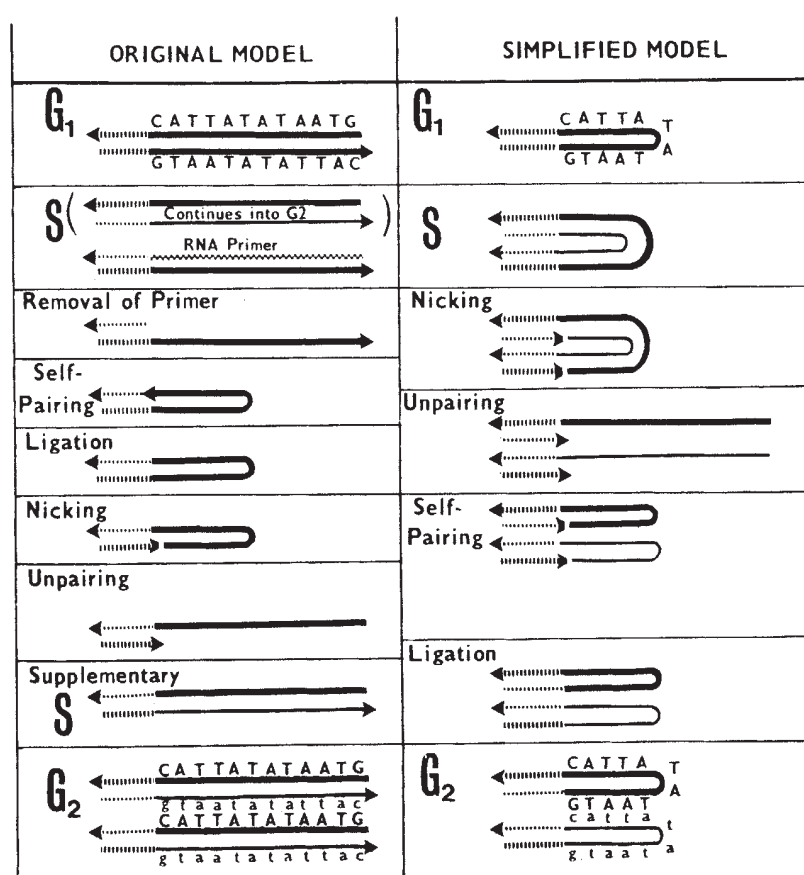


Fig. 1 Two models for the replication of telomeres. —, Old DNA; —, new DNA; ---, RNA. The arrowheads indicate the 3' ends of the phosphate backbone. Capital letters nucleotides of old DNA. Small letters, new DNA. Entire line, the palindrome. Dotted line, the rest of the DNA molecule (of indefinite length).

DR CAVALIER-SMITH REPLIES—I like Bate-man's modification of my model. It implies that a non-replicating eukaryote chromosome is a single, circular self-complementary polynucleotide chain; there are several ways this might be tested. In fact recent evidence indicates that vaccinia virus DNA may have this structure¹. Some other viruses do not, however, and an enzyme system has been isolated² from phage T7 which can cross-link the termini of some of them (T7, T3, T1 and λ) in the ways postulated in my original paper³.

This implies that even these phages have terminal palindromes; they may therefore be able to replicate according to my original model as well as (or instead of) Watson's concatomeric one. Another model involving a terminal palindrome has been independently proposed⁴ for the replication of linear mitochondrial DNA in *Tetrahymena*. But this postulates a palindrome only at one end and therefore requires a circular intermediate, and so seems implausible for eukaryote nuclear DNA.

Department of Biophysics,
School of Biological Sciences,
University of London King's College,
26-29 Drury Lane, London WC2B 5RL, UK

¹ Geshelin, P., and Berns, K. I., *J. molec. Biol.*, **88**, 785-796 (1974).

² Sadowski, P., McGeer, A., and Becker, A., *Can. J. Biochem.*, **52**, 525-535 (1974).

³ Cavalier-Smith, T., *Nature*, **250**, 467-470 (1974).

⁴ Arnberg, A. C., Van Bruggen, E. F. J., Clegg, R. A., Upholt, W. P., and Borst, P., *Biochim. biophys. Acta*, **361**, 266-276 (1974).

Hepatocyte sialoglycoprotein

EVANS¹ reported that the plasma membrane enzyme nucleotide pyrophosphatase is located on the external face of the hepatocyte surface. Based on this result, Evans argues that proposals which suggest cell surface glycosyl transferases function in cell adhesion^{2,3} and in selective binding of asialo-glycoproteins⁴ are less tenable.

As nucleotide pyrophosphatase on the outer membrane surface rapidly cleaves nucleotide sugars and Roseman² has placed an important role for these glycosyl transferase substrates in his theory of cell adhesion, Evans says the proposed mechanism would not be possible. I wish, however, to re-emphasise our results which implicate liver plasma membrane galactosyl transferase in the binding of asialo-glycoproteins⁴. We suggest that this result modifies Roseman's original proposal to the extent that cells remain attached to one another by complexes between glycosyl trans-

ferases and their oligosaccharide products. Thus, the liver recognition of non-reducing terminal galactosyl residues on asialo-glycoproteins does not require any involvement of nucleotide sugars, as the active transferase seems to be galactosyl transferase for which the asialo-protein is a product, and not a substrate.

Evans' result suggests to us that another possible importance for nucleotide pyrophosphatase being localised on the exterior of hepatocytes is that this enzyme may catalyse degradation of external RNA. Clearly a cell must exclude foreign messenger RNA from its cytoplasmic environment, as such a molecule might function in protein synthesis resulting in the eventual transformation of that cell. Such a potent effect of RNA has already been shown to be operative for RNA tumour viruses. Naked RNA itself can be infective⁵, and the addition of other macromolecules which may complex with the RNA and thus protect it from enzymatic hydrolysis enhances infectivity⁶. Results have shown⁷⁻⁹ that liver plasma membrane degrades RNA, and inhibition of membrane phosphodiesterase (nucleotide pyrophosphatase) with EDTA diminishes this activity^{7,9}. A second, well-established marker enzyme for the plasma membrane is 5'-nucleotidase and it also has been shown to be on the outside surface of both liver¹⁰ and leukocyte¹¹ membranes. The 5'-nucleotide products of nucleotide pyrophosphatase would be substrates for 5'-nucleotidase, and indeed liver plasma membrane completely hydrolyses RNA to nucleosides plus inorganic phosphate⁷.

NATHAN N. ARONSON, JUN.
Department of Biochemistry,
The Pennsylvania State University,
University Park, Pennsylvania 16802

¹ Evans, W. H., *Nature*, **250**, 391 (1974).

² Roseman, S., *Chem. phys. Lipids*, **5**, 270 (1970).

³ Roth, S., *Q. Rev. Biol.*, **48**, 541 (1973).

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Matters arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 300 words and the briefest of replies, to the effect that a point is taken, should be considered.

⁵ Gierer, A. and Schramm, G. S., *Nature*, **177**, 702 (1956).

⁶ Seljelid, R., Silverstein, S. C., and Cohn, Z. A., *J. Cell Biol.*, **57**, 484 (1973).

⁷ Yannarell, A. and Aronson, N. N., jun., *Biochim. biophys. Acta*, **311**, 191 (1973).

⁸ Emmelot, P., Bos, C. J., Benedetti, E. L., and Rumke, P. H., *Biochim. biophys. Acta*, **90**, 126 (1964).

⁹ Prospero, T. D., Burge, M. L. E., Norris, K. A., Hinton, R. H., and Reid, E., *Biochem. J.*, **132**, 449 (1973).

¹⁰ Farquhar, M. G., Bergeron, J. J. M., and Palade, G. E., *J. Cell Biol.*, **60**, 8 (1974).

¹¹ DePierre, J. W., and Karnovsky, M. L., *Science*, **183**, 1096 (1974).

DR EVANS REPLIES—Aronson is correct in pointing out that the binding of asialo-glycoproteins by a receptor, possibly a galactosyl transferase present in a liver plasma membrane fraction can occur without a requirement for sugar nucleotides. The involvement of sugar nucleotides in the transglycosylation reaction was postulated by Roseman to account for the de-adhesion process¹ and therefore their hydrolysis by the surface-located nucleotide pyrophosphatase would be a problem. Such proposals, however, must remain tentative until it is demonstrated more clearly that galactosyl and other sugar transferases are indigenous and functional components of the liver plasma membrane, and do not reflect contamination by Golgi membranes.

The plasma membrane fraction used by Aronson was shown to derive mainly from the liver sinusoidal surface² and to be contaminated by Golgi membranes as indicated by morphological markers and high activities of galactosyl and sialyl transferases^{3,4} that we have shown to be absent from the contiguous and bile canalicular face subfractions³. A fuller assessment of a possible role for cell surface galactosyl transferases and associated enzymes in the binding of serum components and in cell adhesion must await further analysis of the specific domains of the surface membrane and their interrelationships with Golgi membranes. Recently, the absence of cell surface galactosyl transferase in a number of cell lines was reported⁵, supporting the general conclusion that this enzyme does not play a role in cellular adhesion, cell recognition and contact inhibition.

Medical Research Council,
National Institute for Medical Research,
London NW7, UK

¹ Roseman, S., *Chem. Phys. Lipids*, **5**, 270-297 (1970).

² Wisher, M. H., and Evans, W. H., *Biochem. Soc. Trans.*, **2**, 407-408 (1974).

³ Wisher, M. H., and Evans, W. H., *Biochem. J.*, (in the press).

⁴ Dewald, B., and Touster, O., *J. biol. Chem.*, **248**, 7223-7233 (1973).

⁵ Deppert, W., Werschan, H., and Walter, G., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 3068-3072 (1974).

reviews

Creative Malady. By George Pickering. Pp. 327+8 plates. (Unwin and Allan: London, 1974.) £5.25.

THE relationship between creativity and illness is one which stimulates the interest of most of us, particularly when an achievement is of the order of genius and seems to involve an impressive destructive action upon the subject's personal life. In this book Sir George Pickering, former Professor of Medicine at London University, attempts to elucidate this peculiar relationship by studying the lives of six famous people: Charles Darwin, Florence Nightingale, Mary Baker Eddy, Sigmund Freud, Marcel Proust and Elizabeth Barrett Browning.

The main hypothesis put forward by Sir George is that creative malady takes two quite distinct forms. In the first, exemplified by Darwin and Florence Nightingale, the illness serves a specific purpose: that of protecting the 'invalid' so that he or she can undertake the immense creative task undisturbed and unmolested. The second kind of creative illness can be observed in the lives of Freud, Proust and Mary Baker Eddy. In these cases productivity followed a period of "great turmoil and torment of mind" which were of sufficient degree to merit the term illness. Freud noted himself that he could not do creative work when he felt well and happy. The last subject in the book, Elizabeth Barrett Browning, unlike the others, showed no obvious connection in her life between creativity and illness.

Sir George gives most of his attention to the first of these types of illness as displayed by Darwin and Florence Nightingale. He has a rare gift for concise and vivid biography and these two lives make fascinating reading. Moreover, he is able to show clearly that the two subjects did indeed use their illnesses as a defensive barrier against forces which would have impinged on their work. (Both of them, for instance, became prostrated by palpitations and other symptoms in the face of intrusion by unwelcome friends, relations or public engagements.)

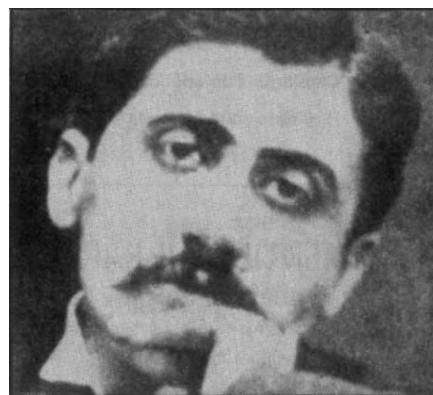
Creative Malady would, however, have been better if Sir George had focused throughout on that theme, for when he turns to other factors in creative illness he underestimates the complexity of the problem and is far less convincing. 'Creativity' and 'illness' are not simple and unambiguous con-

Genius and the mortal afflictions

"Creative genius arises, in a few rare people, out of an early despair of loving . . .

Therein lies the strength and ruthlessness of their drive.

Little wonder they are scarred or ill". Proust (pictured right) was only one of many so afflicted. Review by Peter Lomas



cepts and the two cannot be treated adequately as distinct entities. Take, for instance, Darwin's confessions about himself:

"I have said in one respect that my mind has changed during the last twenty or thirty years. Up to the age of thirty, or beyond it, poetry of many kinds, such as the words of Milton, Gray, Byron, Wordsworth, Coleridge, and Shelley, gave me great pleasure, and even as a schoolboy I took intense delight in Shakespeare, especially in the historical plays. I have also said that formerly pictures gave me considerable, and music very great, delight. But now for many years I cannot endure to read a line of poetry; I have tried lately to read Shakespeare, and found it so intolerably dull that it nauseated me. I have almost lost my taste for pictures or music.

This curious and lamentable loss of the higher aesthetic tastes is all the odder, as books on history, biographies, and travels (independently of any scientific facts which they may contain), and essays on all sorts of subjects interest me as much as ever they did. My mind seems to have become a kind of machine for grinding general laws out of large collections of facts, but why this should have caused the atrophy of that part of the brain alone, on which the higher tastes depend, I cannot conceive."

The impoverishment which Darwin describes could itself be considered either as a form of illness (in the sense of psychological disturbance) or as a state of mind liable to provoke manifest illness. How much impoverishment can a person take before becoming ill? To what extent was Darwin's neurosis caused by conflict between creative drive and the reluctance to pay the heavy price? And what is the origin of a drive so strong that it can, in many cases, wreak such personal havoc? Sir George does not really get to grips with questions of this kind and

that is because, in spite of his admiration for Freud, he places too little emphasis on early individual development. He comes closest to that in describing Proust's relationship with his mother.

Proust was 'in love' with his mother all his life and wrote *A la Recherche du Temps Perdu* only after her death, as if to fill a void. But I suspect that the real void began much earlier. Sir George quotes a moving passage from the great novel in which Proust describes his passionate dependence, as a child, on his mother's all-too-brief goodnight kiss. Sir George writes:

"Why then did Proust write a masterpiece while I have not? A difference of talent no doubt. Moreover, Proust was rich and never had to earn his living. While Proust was able to devote his whole talent to creating his book, I was leading an extremely busy life of practice, teaching, research and public affairs. But there is another great difference. While I was devoted to my mother, as he was to his, and while I had more need of her, since my father died when I was three, my *Recherche du Temps Perdu* would not have lingered on or indeed referred to any incident when my mother withheld the goodnight kiss. If she did so, I have no memory of it whatsoever."

That, I believe, is the crucial factor in an exceedingly complicated and little known phenomenon. Creative genius arises, in a few rare people, out of an early despair of loving. They create an alternative world as a substitute for the world they have lost. Therein lies the strength and ruthlessness of their drive. Little wonder they are scarred or ill.

Sir George may not have written a masterpiece. But he has given us a book which not only contributes to our understanding of creativity but also is a delight to read. □

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Storage diseases

Enzyme Therapy in Lysosomal Storage Diseases. (Proceedings of the Workshop on Cell Biological and Enzymological Aspects of the Therapy of Lysosomal Storage Diseases, Leiden.) Edited by J. M. Tager, G. J. M. Hooghwinkel and W. Th. Daems. Pp. xi+308. (North Holland: Amsterdam and Oxford; American Elsevier: New York, 1974.) Dfl65; \$25.00.

THE identification of the lysosomal enzymes which are lacking in various human, genetically-controlled storage diseases, transformed the approach to this subject. Now that there is a large measure of agreement on the classification and manifestations of these diseases hope is rising that something might be done to ameliorate the conditions of those who suffer from the consequent physical abnormalities, mental retardation, pain and death. Most of the storage defects can be manifested in individual cultured cells which have enzymes missing and in which substrates consequently accumulate. Several of the defects can be identified in cultures of amnion cells, so that prenatal diagnosis is now feasible, which allows the possibility of induced abortion of homozygous children in affected families.

In April 1974 a workshop on the possibilities of enzyme replacement therapy was held in Leiden, and the papers presented were collected for publication in this volume. Many are concerned with the purification and further characterisation of the relevant enzymes and isoenzymes. Others are concerned with *in vitro* complementation, when cultured cells with different defects are fused to form heterokaryons or, remarkably, simply pick up enzymes from each other to attain phenotypically normal characteristics. Since the defect is in the lysosomal system, it may seem relatively easy to restore the missing enzyme, but replacement therapy presents serious difficulties. Liposomes have been used to introduce hydrolases into cells, where they can break down lysosomal contents in model systems. These can protect enzymes from inactivation by antibodies in the circulating blood.

Various attempts have been made to cure deficiency diseases by allotransplantation. In Fabry's disease accumulation of trihexosyl ceramide is often associated with deterioration of kidney function and, according to Desnick, kidney transplantation results in a fall in plasma trihexoside levels and the alleviation of painful crises. Although the missing enzyme, α -galactosidase A, is present in the urine, it is not demonstrable in blood, and the substrate is thought to be filtered from the blood and degraded in the kidney.

Enzymes administered by parenteral injection do not reach the central nervous system, so that a major problem will be to alleviate the effects of storage diseases there. If, however, levels of substrates in the blood could be reduced, there could be some beneficial effect.

The book's usefulness is increased by its rapid publication, made possible because the photo-offset process was used. The standard of reproduction of line illustrations is excellent and even that of half-tone illustrations is acceptable. I suspect that this will soon become the standard way to publish meeting reports.

A. C. Allison

Introducing physics

Solid State Physics. (The Manchester Physics Series.) By H. E. Hall. Pp. xviii + 351. (Wiley: London and New York, September 1974.) \$15.00; £7.50.

THIS fourth book in the Manchester Physics Series is intended for use in undergraduate courses. The author has divided the text into two parts. In the first five chapters he presents a self contained, but elementary, introduction to the basic concepts of solid state physics, which should also be suitable for chemistry students. The early sections include topics such as crystal geometry, lattice vibrations, transport in semiconductors, free electron theory and magnetism. The remainder of the book is aimed towards honours physicists and contains chapters on band theory, X-ray and neutron scattering, thermal conductivity and Fermi surfaces. As a result of this division the formal constructs of reciprocal space and Brillouin zones do not appear until chapter six. The first chapter also flouts convention since the author attempts to explain why solids take up their various lattice structures rather than simply listing the possibilities. Indeed, the book begins with a detailed account of the quantum mechanics of covalent and ionic binding and the author follows this general line of argument throughout. The last few chapters on superconductivity, magnetic ordering and disordered solids are concise, but nevertheless, instructive. The section on the Fermi surface is, however, somewhat spoiled by the unwarranted amount of space (for a book of this kind) which is given to Overhauser's work on possible instabilities of the electron gas.

Each chapter has a useful set of problems associated with it, and answers are provided. I can recommend this as a good, if somewhat unusual, introductory text book. For a full honours course, however, one would probably require a little more experimental and theoretical detail than is contained here.

R. Evans

From metal to insulator

Metal-Insulator Transitions. By N. F. Mott. Pp. xvi+278. (Taylor and Francis: London, September 1974.) £6.50.

In the early days of quantum mechanics a simple model of metals was introduced in which all valence electrons were assumed to be free, or 'nearly' free, when a weak interaction with the static lattice of the positive ions was allowed for. The model predicted that in a perfect crystal the electron states would lie in bands and that each band could accommodate two electrons in every atom. Thus, materials with odd numbers of electrons in each atom, or with occupied and empty bands that overlap, would be good conductors. An insulator could only be changed into a metal (at least at zero temperature) by making its bands overlap. That outstanding prediction survived the test of time. In a real solid, however, the electrons interact with one another and also respond to lattice vibrations and the conditions under which a metal-insulator transition may occur depend to a large degree on the nature of those interactions. The main theme of Professor Sir Nevill Mott's book is to describe the effect of interactions between electrons in inducing magnetic moments and metal-insulator transitions. He reviews the present state of knowledge in the field from both theoretical and experimental standpoints.

The book is divided into six chapters. In the first, the concept of metal-insulator transition is developed within the framework of the model of non-interacting electrons in crystalline and non-crystalline media. The second chapter introduces electron-phonon interaction and exciton formation into the discussion of metal-insulator transition. Chapter 3 deals with the theory of localised magnetic moments in metals, magnetic ordering in non-metallic oxides and metallic ferromagnets, and also outlines the physics of the Kondo effect and its relevance to the main theme of the book.

Chapter 4 covers the metal-insulator transition resulting from correlation. This forms the hard core of the book and includes perhaps its best written passages. It begins with Professor Mott's original argument that at zero temperature a small number of free carriers can always form bound pairs through the long-range Coulomb potential, thereby producing an excitonic insulator. As a consequence, the corresponding metal-insulator transition involves discontinuity in the number of carriers.

Metal-insulator transition can, however, also take place without any reference to the long-range forces. The

Hubbard model, in which only intra-atomic correlation is included, predicts a continuous change in carrier concentration in the region of metal-insulator transition. This theory and its numerous extensions are discussed in some detail. The chapter also describes transitions in highly correlated metals and Wigner and Verwey transitions. The last two chapters apply the ideas of the previous chapter to metal-insulator transitions in transition metal compounds (V_2O_5 , Ti_2O_3 , VO_2 , NiS , and so on) and in disordered systems (doped semiconductors, metal-ammonia solutions).

It must be remembered that although some of the most important contributions to the theory of metal-insulator transition were made many years ago (N. F. Mott, *Proc. Phys. Soc.*, **A62**, 416; 1949) the field is still very much a new one and, as Professor Mott himself points out, there is, among theoretical physicists, no consensus to speak of. Under such circumstances Professor Mott's attempts to give a unified view, embracing a wide variety of experimental and theoretical material, must be highly praised. The book will be a welcome source of up-to-date information (a list of over 500 references to scientific papers and books is also included) to postgraduate researchers in solid state physics. **M. Jaros**

Sequences

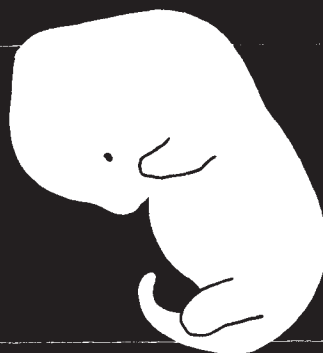
Handbook of Nucleic Acid Sequences. By B. G. Barrell and B. F. C. Clark. Pp. 104. (Joynson-Bruvvers: Oxford, May 1974.) £4.40 cased; £2.50 looseleaf.

This handbook is essentially a comprehensive catalogue of most of the known RNA and DNA sequences so far published and, of necessity, the bulk of the listings refer to the 50 or so tRNA species which have been sequenced. Another attractive target for sequencers are the 5S ribosomal RNAs and although little can yet be said about their function, the primary structures of nine different species of this molecule have been established. Several other low molecule weight (4.5S-6S) RNAs are included as well as the sequences of RNA transcripts from some DNA phages.

A second major section lists all the known sequences from coliphage group I and group III RNAs, and the third section shows the current state of the art of the DNA sequencer and includes a synopsis of DNA restriction enzyme cleavage sites. One would anticipate that the field covered in this section will expand rapidly and it is to be hoped that arrangements are in hand for the regular updating of this collection. **J. Hindley**

Developmental Biology books

1975



Development of the Avian Embryo

A Behavioural and Physiological Study
B. M. FREEMAN and M. A. VINCE

December 1974: 380 pages: 15 pages of plates and 110 line illustrations: hardback: 412115204: £10.10

This book considers the behavioural and physiological development of the avian embryo. After describing the physical conditions needed for development both in incubators and in the wild, the first part goes on to describe the major changes which occur in embryonic physique, posture and activity in the course of incubation. The second part discusses the physiological aspects of development in detail. The book is intended primarily for the research student and the established research worker.

Differentiation and Growth of Cells in Vertebrate Tissues

Edited by G. GOLDSPIK

December 1974: 334 pages: 109 tone and 25 line illustrations: hardback: 412113902: £10.30

This book brings to the fore a relatively neglected part of developmental biology as far as textbooks are concerned; namely the development of the cells in different tissues of the body. There are seven specialised chapters, and each chapter is written by an expert in that field and an all-round picture is given of the biochemical physiology as well as the morphological events associated with the development of that particular tissue. The volume will prove invaluable to postgraduates and advanced undergraduates of biology and medicine.

Cellular Interactions in Animal Development

ELIZABETH M. DEUCHAR

February 1975: 308 pages: 12 pages of plates and 126 line illustrations: hardback: 412130106: £6.50

This book provides a broad and critical survey of the many types of interaction that have been shown to take place between cells during the processes of development in animals.

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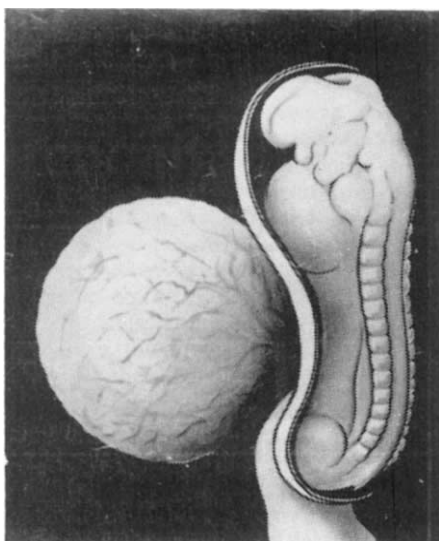
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Film review

Looking at ourselves before birth



A 25 day old embryo, with its connecting stalk and yolk sac.

To describe the development of a human being from ovum to newborn baby in just over half an hour must inevitably mean an extensive compression of the available factual material. In *Ourselves Before Birth* (Macmillan Educational Films) the precis is unfortunately uneven. Ovulation, fertilisation, implantation and the early formation of tissue of a human embryo are described in the first third of the film and the rest of the time is devoted to the growth of the foetus and the development of its blood supply. That imbalance is, however, a major flaw in an otherwise admirable presentation.

As an exposition on foetal growth the film must be one of the best ever made, and the graphics are excellent. But as an educational aid for the study of human embryology in the first few days of life it is disappointing. The men behind the film, Professor W. J. Hamilton and Professor T. W. Glenister, of the Univer-

sity of London, have tried to cram far too much complex information in the first part of the commentary without explaining the technical terms introduced. If the members of an audience already know the meanings of these terms then they have no need to watch the first ten minutes or so of what is a rather superficial treatment; if they do not understand them then they will not be enlightened by this film.

So, for me, the film fell between two stools. As an undoubtedly excellent account of later foetal development it is burdened by technicalities relating to early embryology, which are not explained adequately. There is, however, one marked exception. The implantation of the embryo is beautifully illustrated by the animation which alternates between two and three-dimensional views of the interior of the uterus and the inside of the blastocyst. This technique is most effective—why was it not maintained for perhaps another 10 minutes or so to surmount the hurdle of early tissue formation?

John Wilson

announcements

International meetings

February 14, **Pesticides in Agricultural Control**, London (Mr J. M. Rowell, Society for Chemical Industry, 14 Belgrave Square, London SW1X 8PS, UK).

February 19–20, **Offshore Oil Needs Onshore Fabrication**, Glasgow (Colin Maynard, The Institute of Petroleum, 61 New Cavendish Street, London W1M 8AR, UK).

April 7–11, **Annual Chemical Congress**, York (Dr John F. Gibson, The Chemical Society, Burlington House, London W1V 0BN, UK).

April 9–11, **European Space Research Organisation Tribology Symposium**, Frascati, Italy (ESTEC, Mechanisms and Tribology Section, Structure Division, ESTEC, Domeinweg, Noordwijk, Netherlands).

April 23, **Machine Intelligence**, London (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1X 8QX, UK).

Miscellaneous

Residential course on enzyme and fermentation biotechnology. Organised by the Biochemistry Department at the University of Surrey and the Society of Chemical Industry, the course is aimed at young graduates and experienced workers in the field of technology. Further details from: Dr Alan Wiseman, Department of Biochemistry, University of Surrey, Guildford, UK.

Reports and Publications

Great Britain

Chemistry and Industry Buyers' Guide 1975: Chemicals, Manufacturing Plant and Laboratory Equipment. Pp. 70. (London: The Society of Chemical Industry, 1974.) [2511]

Mycotoxins in Food. By Dr B. Jarvis. (Scientific and Technical Surveys No. 83.) Pp. 20. (Leatherhead: The British Food Manufacturing Industries Research Association, 1974.) [2611]

Department of the Environment. The Welsh Office. Report of a River Pollution Survey of England and Wales 1973, Vol. 3. Pp. xii + 32. (London: HMSO, 1974.) £1.20 net. [2711]

Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 277, No. 1269: On Finite Amplitude Convection in a Rotating Magnetic System. By P. H. Roberts and K. Stewartson. Pp. 287–315. (London: The Royal Society, 1974.) £1.05; £1.10 overseas. [2811]

Technology at Work. Pp. 34. (Cambridge: The Director—Corporate Projects, Pye of Cambridge, Ltd., 1974.) gratis. [2911]

People Without Choice. By Brian Abel-Smith. (Report of the 21st Anniversary Conference of the International Planned Parenthood Federation.) Pp. 68. (London: International Planned Parenthood Federation, 1974.) [2911]

Reshaping Britain: a Programme of Economic and Social Reform. (Broadsheet No. 658.) Pp. 98. (London: PEP/The Social Science Institute, 1974. Orders to Research Publications Services, Ltd., Victoria Hall, Fingal Street, East Greenwich, London, SE10.) £2. [212]

Natural Environment Research Council. Monks Wood Experimental Station—Report for 1972/1973. Pp. 103. (Abbots Ripton, Huntingdon: The Institute of Terrestrial Ecology, Monks Wood Experimental Station, 1974.) 60p. [212]

The Royal Society. Report of Council for the year ended 31 August 1974. Pp. 99. (London: The Royal Society, 1974.) [312]

The British Council. Annual Report 1973/1974. Pp. 107 + 10 photographs. (London: The British Council, 1974.) [312]

The Autonomy of the Broadcasters: Constitution and Convention. By Sir Michael Swann, FRS. Pp. 12. (London: BBC, 1974.) [412]

Science Research Council. Radio and Space Research, January 1971–December 1973. (The Report of the Director of the Appleton Laboratory.) Pp. 83. (London: HMSO, 1974.) £1.25. [412]

Parkinson's Disease. Pp. 24. (London: Office of Health Economics, 162 Regent Street, W1, 1974.) 25p. [512]

Forty-seventh Annual Report of the Agricultural Research Institute of Northern Ireland, 1973/1974. Pp. 47. (Hillsborough, Co. Down: Agricultural Research Institute of Northern Ireland, 1974.) [512]

CEGB Research. No. 1, December 1974. Pp. 36. (London: Central Electricity Generating Board, 1974.) [512]

Other countries

CERN—European Organization for Nuclear Research. CERN 74-20: Selected Physics Data on Neon-Hydrogen Mixtures. Compiled by H. Leutz, F. Schmeissner and H. Weninger. Pp. 175. CERN 74-21: *Radiation Measurements Around the Fermilab 3,000 GeV Main Accelerator*. By M. Awschalom, D. D. Yovanovitch, K. Goebel, K. P. Lambert, J. Ranft and E. Wilson. Pp. 35. (Geneva: CERN, 1974.) [1311]

World Health Organization. Technical Report Series No. 553: Ecology and Control of Rodents of Public Health Importance—Report of a WHO Scientific Group. Pp. 42. (Geneva: WHO; London, HMSO, 1974.) Sw.fr.5. [1311]

Smithsonian Contributions to Zoology, No. 173: *Ostracoda (Myodocopina) of Cape Cod Bay, Massachusetts*. By Louis S. Kornicker. Pp. 20. (Washington, DC: Smithsonian Institution Press, 1974. For sale by US Government Printing Office.) 65 cents. [1311]

Smithsonian Contributions to Zoology, No. 167: *Studies of Neotropical Caddisflies, XVII: The Genus Smicridea from North and Central America (Trichoptera: Hydropsychidae)*. By Oliver S. Flint, Jr. Pp. iii + 65. (Washington, DC: Smithsonian Institution Press, 1974. For sale by US Government Printing Office.) \$1.50. [1411]

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